JOURNAL
OF THE
FISHERIES RESEARCH BOARD
OF CANADA

Volume 29, No. 4

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Fisheries Research Board of Canada
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The Effect of Intermittent Exercise on Carbohydrate Metabolism in Rainbow Trout, *Salmo gairdneri*[1,2]

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ABSTRACT

The effect of intermittent severe exercise of short duration on carbohydrate metabolism was examined using unanesthetized, intact rainbow trout. The levels of muscle glycogen, muscle lactate, blood lactate, and liver glycogen were determined in fish sampled immediately after severe exercise of 3 sec to 5 min, after recovery of 3 min to 60 min, and after re-exercise of 3 sec to 5 min. It appears that rainbow trout are not well adapted to tolerate frequent exercise of short duration.

INTRODUCTION

There have been many studies of the relationship between severe exercise and concomitant changes in carbohydrate metabolism in fish (von Buddenbrock, 1938; Secondat and Diaz, 1942; Black et al., 1961; Caillouet, 1964). In almost all of these studies the fish were continuously exercised for 15 min. Exercise of this duration always elevated muscle and blood lactate levels and decreased muscle glycogen levels.

There have been very few studies of the effects of discontinuous or intermittent severe exercise on carbohydrate metabolism in any intact animal, and none discovered in the literature on fish to date. Christensen (1962) demonstrated that man can do an optimum amount of exercise in a given time by alternate exercise and rest periods of 30 sec. His results showed that continuous exercise for 9 min elevated the level of blood lactate from 7 to 150 mg/100 ml, whereas discontinuous exercise for 60 min (alternate exercise and rest periods of 30 sec) elevated the level of blood lactate to only 20 mg/100 ml.

The present study compares the effects of continuous and discontinuous exercise on carbohydrate metabolism in rainbow trout. The levels of severe exercise studied approximate the levels of severe exercise which rainbow trout probably experience in their natural environment. An increase in the concentration of muscle lactate is taken to indicate that the oxygen supply is not meeting the demand. The change in level of blood lactate is indicative of the rate of release of lactate from the muscle to the blood. The change in levels of glycogen in the muscle and liver indicate the rate of utilization of energy reserves.

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[1]Received for publication September 3, 1965.
[2]This study was supported by a Grant-in-aid from the National Research Council of Canada.
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J. Fish. Res. Bd. Canada, 23(4), 1966.
Printed in Canada.

METHODS

Rainbow trout (*Salmo gairdneri*), 1½ years old, were reared from eggs obtained from a commercial trout hatchery in McLeary, Washington. They were raised at the Summerland Trout Hatchery operated by the British Columbia Department of Recreation and Conservation, Fish and Game Branch. Trout were fed twice daily with pelleted fish food (J. R. Clarke, Utah). The water temperature ranged from 10.5 to 11.5 C. At the outset of the experiment furunculosis was prevalent. The fish were treated successfully with sulfamerazine given with the food so that diseased fish were not used in the experiment. Fish were transferred from outside ponds to standard hatchery troughs at least 2 days prior to experiment. Fish were not starved before the experiment, but were kept covered to reduce the effect of external stimulation.

The methods used to exercise the fish, allow them to recover, and to obtain blood samples were those described by Black (1955, 1957a, and Black et al., 1962). The methods used to obtain tissue samples were those described by Connor et al. (1964). Each fish was sampled only once, blood and tissue samples being obtained from separate fish. Lactate levels were determined colorimetrically on deproteinized filtrates of blood and muscle homogenates by the method of Barker and Summerson (1941). Glycogen was determined colorimetrically by the anthrone method (Carroll et al., 1956) on KOH digests of muscle and liver which were diluted to known volume. Times to obtain and prepare blood and tissue samples are given in Table I.

RESULTS

Muscle Glycogen

The effects of exercise, recovery, and re-exercise are illustrated in Fig. 1–5. Exercise of even the shortest duration caused an immediate decrease in levels of muscle glycogen. Approximately 50% was utilized during 30 sec exercise, and 80% during 5 min exercise. Changes in the level of muscle glycogen during recovery of 3–60 min were negligible. Re-exercise further lowered the level of muscle glycogen.

Table I. Time to obtain and prepare blood and tissue samples. Blood and tissue samples were obtained from separate fish. All times are given in seconds.

Sample		*n*	Mean ± SD
Blood	To catch and position fish	305	13.7 ± 4.1
	To withdraw blood sample	305	31.4 ± 23.9
Tissue	To catch fish	222	2.9 ± 1.8
	To kill fish	222	2.8 ± 1.2
	To remove muscle sample and place in dry ice and ethanol	222	10.4 ± 2.6
	To remove liver and place in dry ice and ethanol	62	14.1 ± 6.4

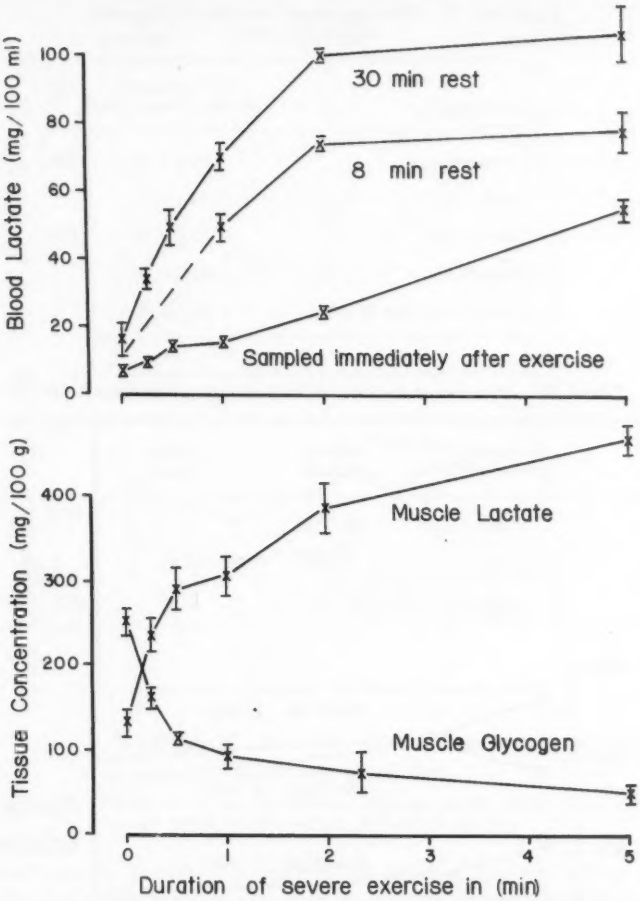

FIG. 1. Changes in the levels of muscle glycogen, muscle lactate, and blood lactate during 5 min severe exercise. Each point represents the mean of at least six samples from separate fish (mean ± standard error).

LIVER GLYCOGEN

Changes in levels of liver glycogen were negligible during exercise, recovery, and re-exercise. The levels of liver glycogen during 5 min severe exercise appear in Table II, and an analysis of variance of these data appears in Table III.

MUSCLE LACTATE

The effects of exercise, recovery, and re-exercise are also illustrated in Fig. 1–5. Exercise of even the shortest duration caused an immediate increase

TABLE II. Effect of severe exercise on the levels of liver glycogen.

Condition	Liver glycogen ($g/100\ g$) mean ± SD
Unexercised	4.90±1.40
Chase 1 min	4.54±2.11
Chase 2 min	4.83±1.30
Chase 5 min	5.21±1.46
Chase 5 min, rest 30 min	3.36±0.88

TABLE III. Analysis of variance of liver glycogen data from Table II.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F	$F_{0.05}$
Total	38	95.7162			
Treatment	4	15.8475	3.96	1.69	2.65
Error	34	79.8687	2.35		

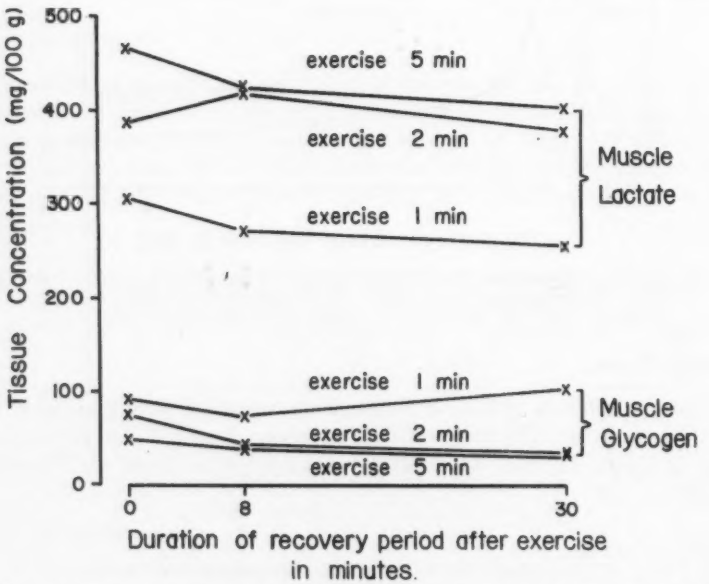

FIG. 2. Changes in the levels of muscle glycogen and muscle lactate during recovery from severe exercise of 1, 2, and 5 min.

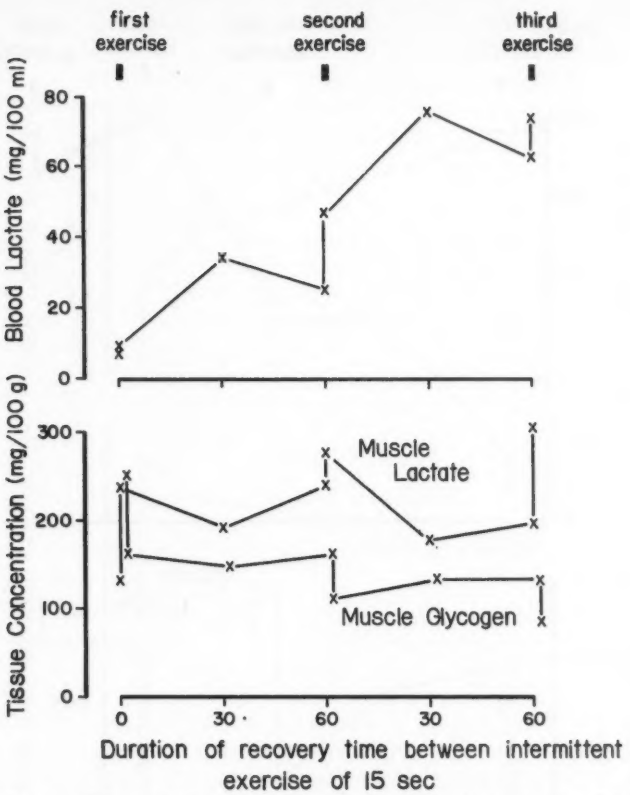

FIG. 3. Effects of 15 sec intermittent exercise on the levels of muscle glycogen, muscle lactate, and blood lactate.

in levels of muscle lactate. Changes in the level of muscle lactate during recovery after exercise were slight, and re-exercise further elevated the levels of muscle lactate. There was an upper limit (approximately 470 mg/100 g) which muscle lactate did not exceed.

BLOOD LACTATE

The effects of exercise, recovery, and re-exercise are illustrated in Fig. 1, 3–5. Exercise of even the shortest duration caused a rapid increase in the levels of blood lactate. The level of blood lactate continued to rise during recovery after exercise, and re-exercise further elevated the blood lactate levels.

DISCUSSION

MUSCLE GLYCOGEN

The level of muscle glycogen in the unexercised condition was slightly higher than the levels reported by others for *Salmo gairdneri* (Miller et al.,

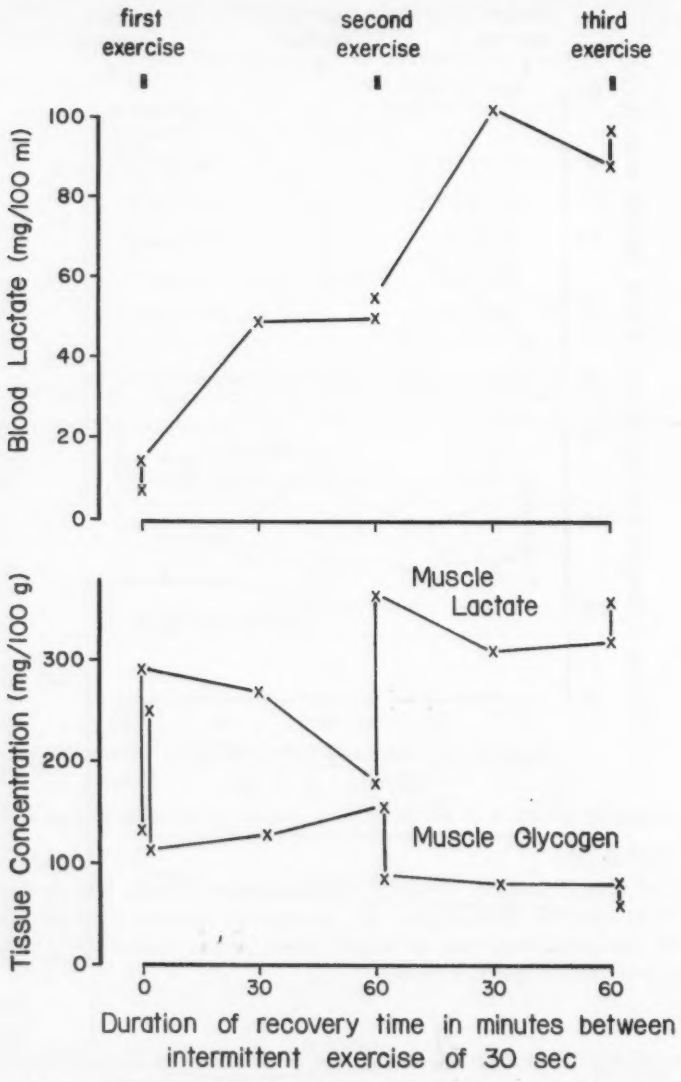

FIG. 4. Effects of 30 sec intermittent exercise on the levels of muscle glycogen, muscle lactate, and blood lactate.

1959; Black et al., 1960) and is about one-quarter of that reported for mammals (West and Todd, 1961).

Depletion of muscle glycogen during exercise in fish has been studied by Miller et al. (1959) and by Black et al. (1960). Both Black and Miller observed that 50% of the muscle glycogen was depleted during 2 min exercise, and

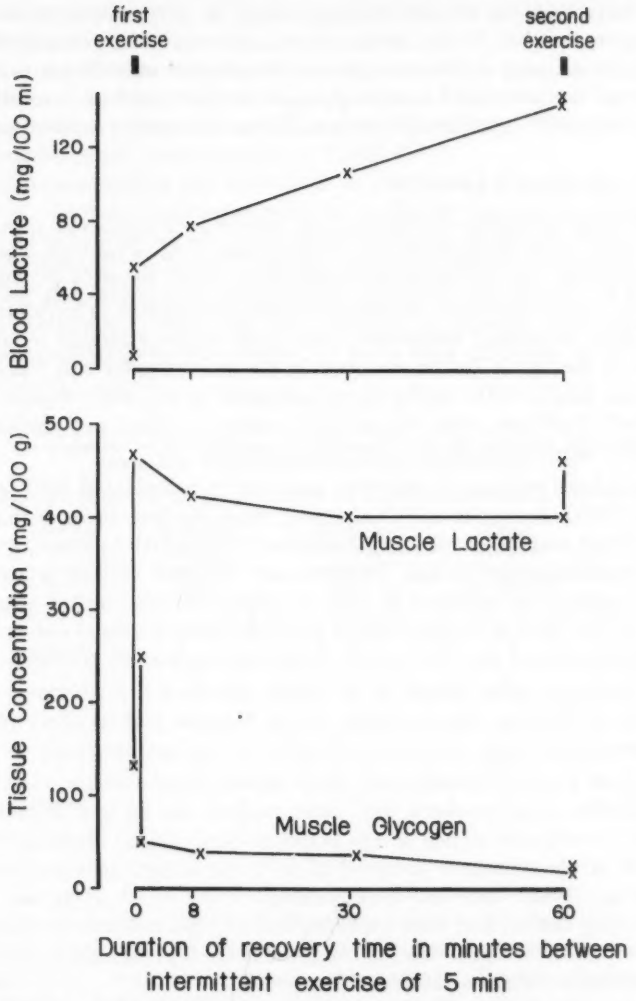

FIG. 5. Effects of 5 min intermittent exercise on the levels of muscle glycogen, muscle lactate, and blood lactate.

80% was depleted during 15 min exercise. In the present study 50% was depleted during 30 sec exercise, and 80% during 5 min exercise. Part of the discrepancy is possibly due to the fact that the level of muscle glycogen in the unexercised condition was higher in the present study than that reported by Black or by Miller.

Resynthesis of muscle glycogen after exercise is a slow process in fish. Black et al. (1962) observed that the level of muscle glycogen in rainbow trout

severely exercised for 15 min did not return to pre-exercise levels during a 24-hr recovery period. In the present study, the resynthesis of muscle glycogen was negligible during a 60-min recovery period after only 15 sec exercise. The slow rate of resynthesis of muscle glycogen in fish could be a result of either enzyme inhibition by lactic acid or insufficient circulation to the muscle.

Muscle and Blood Lactate

The level of muscle lactate in the unexercised condition is much more variable than that of blood lactate, but is similar to other values reported in the literature for fish (Ritchie, 1927; Nakatani, 1957; Black et al., 1962; Scholander et al., 1962). Levels of muscle lactate do not appear to have been studied in any intact terrestrial vertebrate. The level of blood lactate in unexercised condition is similar to values reported in the literature for fish (Secondat and Diaz, 1942; Black 1955, 1957a, b, c; Leivestad et al., 1957; Heath and Pritchard, 1962; Caillouet, 1964; Dean and Goodnight, 1964), and similar to those reported for man (Bock et al., 1932; Bang, 1936).

The lactate response to exercise was first demonstrated by Fletcher and Hopkins (1907). Secondat and Diaz (1942) were the fisrt to show that exercise elevates blood lactate in fish; and Nakatani (1957) first to show that exercise elevates muscle lactate in fish. Fletcher and Hopkins also proposed that the failure of muscle to contract in spite of adequate stimulation after exercise was due to the level of muscle lactate reaching some maximal value. Meyerhof (1920) demonstrated that the source of lactate was muscle glycogen.

In Kamloops trout, Black et al. (1962) observed that the level of lactate was higher in females than in males during exercise and recovery. No consistent differences between sexes were observed in the present study.

Maximal levels of muscle and blood lactate after exercise vary from species to species. Most workers who have studied the lactate response in fish have used 15 min continuous severe exercise. Bang (1936) observed that maximal levels of blood lactate occurred after 1 min severe exercise in man. Anderson et al. (1960) observed that maximal levels of blood lactate occurred after sprinting 400 m, and that sprinting any further failed to produce further increases in blood lactate levels. In the present study, maximal levels of muscle and blood lactate occurred after 5 min exercise.

That the level of blood lactate continues to increase during recovery after exercise has been noted by others (Secondat and Diaz, 1942; Nakatani, 1957; Black et al., 1962). Black (1957a) observed that blood lactate levels continued to increase for 3.5 hr after 15 min exercise in rainbow trout, whereas the delayed increase lasted only 2–5 min in man (Anderson et al., 1960). The prolonged delayed increase of blood lactate after exercise in fish is probably due to inadequate circulation to the muscle.

A major difference between fish and mammals is the slowness with which the level of blood lactate returns to pre-exercise levels after severe exercise. Alpert and Root (1954) showed that 40% of lactate produced by severe exer-

cise in man was utilized in 10 min; whereas Black et al. (1962) showed that in trout 40% was utilized in approximately 8 hr.

There are very few studies of intermittent severe exercise in man, and apparently none in fish. The results of a study of the effect of intermittent exercise on the level of blood lactate in man by Christensen (1956) are compared with the results of the present study in Table IV.

Christensen's results are explained on the basis of myoglobin (Astrand et al., 1960). That is, myoglobin provided sufficient oxygen for 30 sec severe exercise, and that only 30 sec recovery time was required to reload the myoglobin with oxygen. An explanation of the large difference between the response in fish and man is difficult since myoglobin is also present in fish (Matsuura and Hashimoto, 1956). The slow recovery is probably best explained by inadequate circulation to the muscle, or by a slow diffusion of lactate from muscle to blood.

TABLE IV. A comparison of the resistance to fatigue between fish and man during intermittent exercise as indicated by the level of blood lactate.

Animal	Duration of exercise (*min*)	Duration of rest period (*min*)	Duration of total exercise (*min*)	Blood lactate (*mg/100 ml*)	Reference
Man	resting		0	9	Christensen
	½	½	30	20	"
	2	2	30	95	"
	9	0	9	150	"
Fish	resting		0	7	Present study
	½	60	1.5	97	" "

The fate of lactate produced by severe exercise has been the subject of much study. Eggleton and Evans (1930) perfused frog muscle with lactate and observed no increase in muscle glycogenesis. Recent observations by Drury and Wick (1956, 1958) showed that very little lactate was resynthesized into glycogen in the intact rabbit, and most of the lactate was expired as carbon dioxide. They also observed that lactate was oxidized faster than glucose and suggested that lactate could act as a quick fuel in emergencies.

Relationship Between Glycogen and Lactate in Muscle

To demonstrate the relationship between lactate and glycogen in muscle, a regression analysis was done on the data from 73 fish which were sampled immediately after exercise. The correlation coefficient ($r = -0.70$) indicated that the relationship between muscle lactate and glycogen is highly significant.

TABLE V. Sample size, mean, and standard deviation for each of the variables studies. Blood and tissue samples were obtained from separate fish.

Exercise condition					Muscle samples							Blood samples				
						Body weight		Lactate		Glycogen			Body weight		Lactate	
Chase (*min*)	Rest (*min*)	Chase (*min*)	Rest (*min*)	Chase (*sec*)	*n*	(g) mean ± SD		(*mg/100 g*) mean ± SD		(*mg/100 g*) mean ± SD		*n*	(g) mean ± SD		(*mg/100 ml*) mean ± SD	
Unexercised					9	221	49.2	132	49.7	251	48.8	8	310	53.0	7	4.1
1					9	223	27.0	306	69.4	92	41.3	8	275	69.5	15	3.6
2					9	255	29.4	387	88.1	74	75.4	9	252	56.9	24	6.2
5					8	260	64.8	468	50.7	49	15.7	9	219	27.3	55	9.5
1	3				9	245	47.5	362	107.4	46	29.7	9	252	55.0	43	7.7
2	3				9	220	40.6	400	90.0	49	43.3	9	266	38.0	54	8.6
5	3				9	213	48.4	394	85.6	33	43.7	9	256	57.3	74	14.9
1	8				9	242	49.5	272	75.9	73	56.3	9	248	54.6	49	11.5
2	8				9	270	54.4	418	71.0	43	40.0	9	267	44.4	74	5.3
5	8				9	223	34.3	424	62.5	38	45.6	9	274	39.3	78	16.7
1	30				9	207	46.3	256	55.2	103	76.1	9	287	93.6	70	12.9
2	30				9	244	59.9	380	132.7	34	22.8	9	233	53.5	100	7.1
5	30				9	230	76.5	402	91.1	35	24.5	9	260	47.0	107	25.3
5	60				6	303	62.2	403	41.0	19	18.6	6	301	61.1	144	27.6
1	3	1										6	359	40.2	49	5.4
2	3	2										6	272	29.2	79	10.3
5	3	5										6	336	100.5	86	6.7
1	8	1										6	318	55.0	64	5.1
												6	298	49.7	86	7.6

2	8	2										6	298	49.7	86	7.6
5	8	5										6	356	59.5	98	10.4
1	30	1										6	293	34.9	86	14.1
2	30	2										6	349	71.8	115	14.7
5	30	5										6	290	77.3	120	19.0
5	60	5			6	275	43.6	462	42.2	24	11.0	6	294	41.1	148	16.5
(sec)	*(min)*	*(sec)*	*(min)*	*(sec)*												
3												7	247	33.6	9	1.9
3	30											8	286	55.7	24	4.5
3	60											6	265	38.4	19	17.5
3	60	3										6	262	53.3	16	6.9
15					6	259	42.8	236	51.0	161	31.2	6	261	33.8	9	2.8
15	30				6	306	53.3	193	20.7	148	64.0	6	306	56.9	34	8.0
15	60				6	304	51.0	240	114.5	162	30.3	6	295	40.9	25	9.2
15	60	15			6	243	58.0	276	56.0	111	62.9	6	227	46.2	47	20.5
15	60	15	30		6	251	50.4	177	40.8	134	42.2	5	289	61.3	76	5.8
15	60	15	60		6	302	44.0	196	95.9	132	30.5	7	301	25.1	63	20.1
15	60	15	60	15	6	289	42.9	304	83.7	83	44.0	6	298	40.2	74	23.0
30					6	287	65.5	291	61.3	113	12.5	7	308	36.3	14	3.0
30	30				6	263	45.7	271	77.3	127	56.2	6	270	34.9	49	11.1
30	60				6	256	62.6	179	32.0	157	38.5	6	258	58.8	50	15.6
30	60	30			6	253	39.6	366	70.7	85	21.1	6	300	27.1	55	12.1
30	60	30	30		6	294	53.5	309	36.6	81	25.7	6	308	58.3	102	15.5
30	60	30	60		6	304	19.8	320	68.9	81	23.6	6	274	42.2	88	13.1
30	60	30	60	30	6	268	68.3	358	36.1	59	11.6	6	292	40.3	97	19.1

The observed relationship (the calculated regression line) is compared with the theoretical relationship in Fig. 6 assuming that all of the glycogen appears as lactate (i.e. 162 mg of muscle glycogen appear as 180 mg of muscle lactate). The slopes of the two lines are not significantly different ($P < .10$) which indicates that, in fact, all of the glycogen does appear as lactate.

The slowness of recovery can best be explained by the inadequacy of the circulation to the muscle. This temporary inadequacy may result from a circulatory shunt which retards the movement of lactate from muscle to blood through vasoconstriction of the vessels which supply the muscle. Although it appears that rainbow trout are not well adapted to tolerate severe exercise, it is possible that the slowness of recovery of lactate levels is associated with an adaptation which enhances the rate of metabolism during emergencies at the cost of slow recovery.

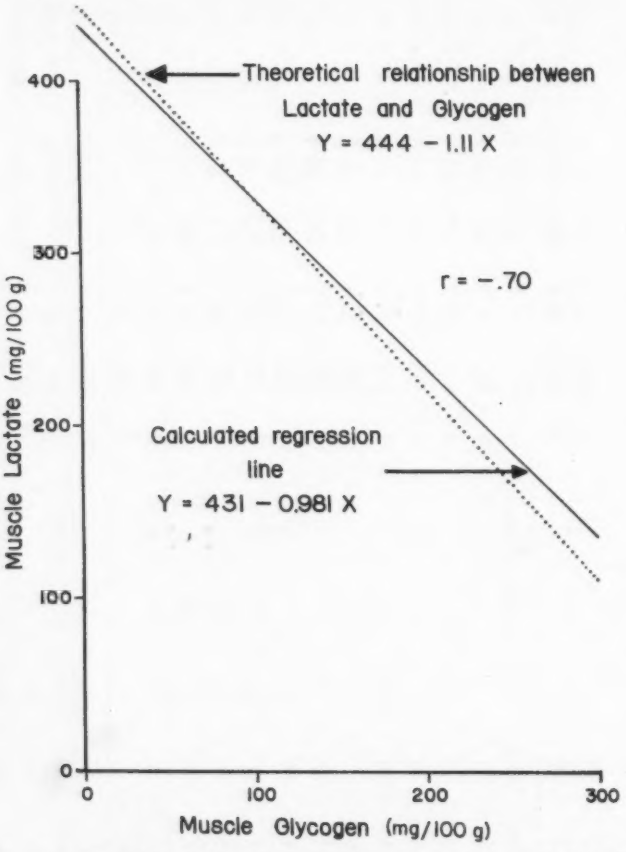

FIG. 6. The relationship between muscle glycogen and muscle lactate in 73 fish sampled immediately after exercise.

SUMMARY

1. The effects of intermittent exercise of short duration (3 sec to 5 min) on carbohydrate metabolism were studied in rainbow trout.
2. Thirty seconds severe exercise caused a 50% depletion of muscle glycogen, a two-fold increase in the level of muscle lactate, and a two-fold increase in the level of blood lactate.
3. Five minutes severe exercise caused an 80% depletion of muscle glycogen, a 3.5-fold increase in the level of muscle lactate, and a 10-fold increase in the level of blood lactate.
4. Changes in levels of muscle glycogen and muscle lactate were negligible during 60 min recovery from severe exercise. Blood lactate levels continued to increase during recovery after severe exercise.
5. The effects of re-exercise were almost additive. That is, re-exercise caused a further decrease in muscle glycogen levels and a further increase in muscle and blood lactate levels.
6. Levels of liver glycogen did not change significantly during exercise recovery, or re-exercise.
7. No mortalities occurred as a result of severe exercise. No consistent effects of sex, weight, catch time, or sample time on glycogen or lactate levels were noted.

ACKNOWLEDGMENTS

Assistance for this project was provided by the National Research Council of Canada through grant T-7 to E. C. Black. The Fish and Game Branch of the British Columbia Department of Recreation and Conservation provided and maintained the experimental animals. Thanks are also due to the following for technical assistance: Mr D. John Bosomworth, and Mr S. J. Tredwell for assistance in sampling and analysis of lactate; Miss G. Docherty for analysis of glycogen; Mr K. Henze of the Department of Physiology, University of B.C., and Miss J. C. Willcox for assistance in preparation of the manuscript. We are also indebted to Dr J. R. H. Dempster and the staff of the Computing Center at U.B.C. for assistance in the calculation of statistics. Drs D. J. Randall and J. R. Brett are thanked for useful criticisms of the manuscript.

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Deposition of Dimethyl-β-Propiothetin in Atlantic Cod During Feeding Experiments[1]

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ABSTRACT

Dimethyl-β-propiothetin (DMPT), associated with dimethyl sulphide (DMS) in the "blackberry" problem in cod from Labrador, was absorbed from the digestive system when fed to laboratory-held cod. Uptake of DMPT by blood and deposition in tissues were readily detected when the intake level exceeded 10 mg DMPT/day. No obvious amount of DMS was formed from the DMPT. The deposits of DMPT were rapidly removed from flesh when intake ceased. Open cooking (baking) at high temperatures of cod flesh containing DMPT completely destroyed DMPT with no deleterious effects. Cooking at lower temperatures (boiling) probably only partly destroyed DMPT as objectionable taste and odour effects were generally observed. During freeze-drying DMPT was partly destroyed.

INTRODUCTION

The odour associated with Atlantic cod (*Gadus morhua*) taken inshore in Labrador waters in late June and early July has been identified as dimethyl sulphide (DMS) (Sipos and Ackman, 1964). This volatile material has been shown to be a breakdown product of dimethyl-β-propiothetin (DMPT), which occurs widely in multicellular marine algae (Challenger, 1959). This thetin has now also been shown to be probably present in a number of phytoplanktonic unicellular marine algae (Ackman et al., 1965).

In the North Pacific a similar problem with chum salmon (*Oncorhynchus keta*) has been examined by Motohiro (1962). A DMS odour (described as a "petroleum" odour) was reported in heat-processed canned salmon taken from mid June to the end of June. Motohiro found DMPT in the bodies, livers, and in the stomach contents, which consisted chiefly of the pteropod *Limacina helicina* at the particular time of year. A localized occurrence of fish condemned from feeding heavily on this pteropod in Hecate Strait in 1964 has recently been reported (Lippa, 1965).

The cod in the Labrador area feed heavily on zooplankters, the typical appearance of the stomach contents giving rise to the local name blackberry. It has not yet been shown that *L. helicina* is the sole zooplankter species involved. Periodically the DMS odour is observed through the Strait of Belle Isle along the north shore of the Gulf of St. Lawrence and also down the northeast coast of Newfoundland, where the term black feed is applied. This distribution corresponds to the Labrador current, and *L. helicina* has been suggested as an indicator of water of arctic origin (Kielhorn, 1952). In Norway

[1]Received for publication September 13, 1965.

J. Fish. Res. Bd. Canada, 23(4), 1966.
Printed in Canada.

a similar feed and odour problem occurs under the descriptive name of gunpowder, and the odour problem is also known in Greenland fisheries.

The Canadian areas involved have traditionally been producers of salt fish and the transient DMS odour has been of no importance. With the potential development of a frozen fish industry it has been essential to explore the possibility that DMPT may be deposited in cod flesh, to ascertain the intake levels for such deposition, to determine if such deposits persist after intake ceases, and to ascertain the effects of possible deposits on commercial utilization of cod from this area.

Blackberry in Labrador waters is not necessarily an annual occurrence, and although it was a serious problem in the 1963 fishery no difficulties were reported in 1964 and 1965. Samples from a recent fishery could not, therefore, be obtained for study and the present report is of preliminary findings from laboratory experiments on holding and feeding cod with DMPT. The exploratory nature of the work and certain difficulties in holding the cod required changes in procedure as the work progressed. These are reported as a guide to further work and to draw attention to what may become a problem in fisheries in the Labrador area. It has, however, been possible to establish that DMPT deposition in cod flesh does occur and is roughly in proportion to intake, to determine an approximate minimum intake for deposition, to show that DMS is not necessarily derived from ingested DMPT, that DMPT is rapidly eliminated when intake ceases, and finally that DMPT in cod flesh can have objectionable taste effects in cooking at moderate temperatures.

EXPERIMENTAL

Holding and Feeding

Fish were obtained locally (Terence Bay, N.S.) by handlining and transported to the laboratory in Halifax by tank truck. After 48 hr in a large live-holding tank, selected fish from those surviving were transferred to small tanks for holding and feeding. These tanks, and the details of the running seawater supply, have been described by Stewart and Power (1963).

As the fish did not eat if the water temperature rose above 9 C (late summer) or fell below 4 C (late winter), it was necessary to install a heating system in the water feed line to raise the water temperature in the holding tanks during the winter. At a flow rate of 6 gal/min (net for six tanks) an installation of 7.5 kw (Calrod heaters, General Electric Co.) raised the water temperature from 3 to 6–7 C.

When one fish was held per tank the mortality rate was high and some otherwise healthy fish refused to eat even after several weeks of fasting. The food offered consisted of chunks (average 12 g each) sawed transversely from frozen herring obtained locally. A fresh lot of fish was obtained and four fish placed in each tank. In the course of 3 weeks there was some natural mortality and at the end of this period nonfeeding fish were removed. The surviving fish were sorted to give two readily distinguishable fish per tank excepting for fish 1A and 1B (Table I), which had individual tanks.

TABLE I. Experimental conditions, DMPT intake of cod, and free DMPT found in certain tissues.

Fish No.	Fish length (*cm*)	Weights on sacrifice (*g*)					Feeding period (*days*)	Time from last DMPT feeding to killing	DMPT contents of capsules (*mg*)
		Whole	Liver	Stomach and contents	Intestines and contents	Fillets as cut			
1A	NR	NR	NR	NR	NR	NR	12	24 hr	10
1B	NR	NR	NR	NR	NR	NR	6	48 hr	10
2A	57	1706	25	23	70	377	15	48 hr	50
2B	48	1045	30	13	95	245	15	48 hr	50
3A	41	523	7	15	4	102	20	48 hr	10
3B	56	1324	75	47	14	294	20	48 hr	10
4A	48	1285	86	22	5	286	10	12 days	10
4B	48	1075	87	15	12	238	10	12 days	10
5A	59	1500	21	NR	NR	272	37	48 hr	50[c]
5B	57	1460	60	NR	NR	357	37	48 hr	50[c]
6A	45	760	28	NR	NR	159	37	48 hr	50[c]
6B	53	1110	43	NR	NR	264	37	48 hr	50[c]
7A	50	970	23	NR	NR	208	37	48 hr	50[c]
7B	58	1600	79	NR	NR	278	37	48 hr	50[c]
8A	72	3100	110	NR	NR	900	10	48 hr	50[d]
8B	49	1050	40	NR	NR	260	10	48 hr	50[d]
9A	88	6140	200	NR	NR	1640	10	48 hr	50[d]
9B	53	1300	55	NR	NR	460	10	48 hr	50[d]

DMPT intake (*mg*)		DMPT in sample analysed (*mg/g*)				Estimated total[b] DMPT in fish (*mg*)	Per cent deposited DMPT (from estimated total)
Observed	Total intake per tank divided between two fish in proportion to observed intake	Fillets	Stomach and contents	Intestines and contents	Blood[a]		
110	110	NA	0.160		NA	....	....
170	170	0.002	0.019		NA	....	....
500	1400	0.262[e]	0.070	0.238	0.017	384	27
200	550	0.109[e]	0.041	0.111	0.011	95	17
NR	300	0.025	0.690[f]	0.030	NA	16	5
NR		0.006	0.014[f]	0.003	NA		
NR	280	0.002	0.004[g]	0.001	NA	4	1
NR		0.002	0.002[f]	0.001	NA		
150	250	0.001[e]	NA	NA	0.001	....	....
300	400	0.040	NA	0.05[h]	0.001	....	....
800	1200	0.570	NA	NA	0.150	....	....
150	200	0.170[e]	NA	NA	0.003	....	....
500	750	0.140[e]	NA	NA	0.007	....	....
600	950	0.090	NA	NA	0.002	....	....
1150	1150	0.302	NA	NA	NA	772	67
1250	1250	1.075	NA	NA	NA	876	70
1400	2100	0.270[e]	NA	NA	NA	1344	63
300	500	0.239	NA	NA	NA	239	48

NR, not recorded; NA, not analysed.
[a]Per millilitre of serum.
[b]Omitting stomach, intestine, and blood DMPT.
[c]In last two feedings herring had two capsules in each chunk.
[d]In all feedings herring had two capsules in each chunk.
[e]Fillets used in cooking experiments.
[f]Stomach contents slightly digested.
[g]Stomach contents nearly digested.
[h]Feces expressed from anus.

In feeding experiments, six chunks of herring were placed in each tank and the activity of the fish observed for 15 min. Usually at least one fish ate immediately and then ceased feeding. The more voracious fish often fed more lightly at the next feeding (after 2–3 days), but ate voraciously at the subsequent feeding. The herring chunks were left 24 hr, removed, and counted. The herring chunks consumed during the period of nonobservation (excepting with fish 1A and 1B) were assumed to be eaten by the initially less interested fish, and when these were killed this was generally supported by the stomach contents.

DMPT Administration

The inner halves of No. 5 U.S.P. standard gelatin capsules were tightly packed with dry DMPT chloride (obtained from Mann Research Laboratories, Inc., New York, N.Y.; or California Corp. for Biochemical Research, Los Angeles, Calif.) or a 3:1 blend of DMPT chloride and fish flour. The capsule halves were prepared in large lots and stored in a desiccator over calcium chloride. A few random samples were checked to verify that blending and packing had been adequate. The capsule halves of blend were recorded as 10 mg of free DMPT per capsule half and the capsule halves of pure DMPT chloride as 50 mg free DMPT. The capsule halves were inserted, open end inwards, deeply and snugly into holes drilled in frozen herring chunks. Preliminary experiments had indicated that in general cod held in 6 C water effectively digested herring chunks in 48 hr, including the capsule halves and contents. Complete capsules were virtaully intact under the same conditions. It had previously been shown that local cod and herring were free of DMPT (Ackman and Dale, 1965).

Sacrifice and Analysis

Fish were removed from the tanks at suitable times and killed immediately by a blow on the head. They were then taken to a cold laboratory (2 C), gutted and filleted, and data recorded as noted in Table I. Blood samples were collected by severing the tail artery.

For fish 1A to 4B samples were analysed as soon as the fish were killed. For fish 5A to 7B samples (excepting blood) were held in frozen storage at −20 C for 4 months prior to analysis. Blood samples were allowed to coagulate and aliquots taken from the serum. For fish 8A to 9B samples were analysed immediately. Livers and some fillets from these fish were stored at −20 C for later examination. Samples, fresh or frozen, were processed in the cold laboratory and were blended 1:4 or 1:2 with distilled water containing about one-quarter ice. The blended materials were decanted into a covered beaker and allowed to stand until a clear layer appeared on the bottom. Usually, this required 15–30 min. Aliquots of the clear layer alone, whenever possible, were removed for analysis.

Analyses for DMS and DMPT were carried out by gas–liquid chromatography as described by Ackman and Dale (1965). For DMS a 7-ml aliquot of the sample in a vial was screwed into position in the reactor, warmed to room temperature with a warm water bath, pressurized with carrier gas and agitated for 2 min. Injection of this "head space" gas normally gave a chromatogram showing one or more pronounced peaks arising from contamination through exposure to room air containing solvent vapors. These were greatly reduced for the subsequent DMPT determination on the same aliquot of sample by several successive pressure purges of the vial with carrier gas while agitating the sample. Injection of concentrated alkali solution with 2 min for reaction as described by Ackman and Dale (1965) then gave DMS for gas chromatographic estimation of the DMPT present. With samples very rich in DMPT a smaller aliquot of blend was used with the addition of distilled water to keep the total volume at 7 ml. In the results (Tables I and II) the figures of 0.001 mg/g sample are nominal and do not necessarily indicate

TABLE II. DMPT levels found in cod livers before and after frozen storage for various periods (mg/g tissue).

Fish No.	When killed	After storage at −20 C for			
		2 weeks	5 weeks	8 weeks	16 weeks
2A	0.152	...	...	...	...
2B	0.180	...	...	...	...
3A	0.004	...	...	...	...
3B	0.004	...	...	...	...
4A	0.002	...	...	...	...
4B	0.003	...	...	...	...
5A	...	...	...	...	0.001
5B	...	...	...	...	0.001
6A	...	...	...	...	0.001
6B	...	...	...	...	0.001
7A	...	...	...	...	0.006
7B	...	...	...	...	0.001
8A	0.430	...	...	0.37	...
8B	0.280	...	...	0.30	...
9A	0.270	0.220	0.056	0.015	...
9B	0.002	...	...	0.001	...

DMPT. The effect of concentrated alkali on fish tissue is such that several trace peaks are normally observed, one of which has the retention time of DMS. The figure of 0.001 mg/g is that for this peak, which may be DMS from a source other than DMPT or another material altogether. This figure is also the effective limit of sensitivity of the method as employed without concentration steps. Higher levels of DMPT may be taken as significant. Data are given to three figures solely for comparative purposes. The actual order of accuracy is dependent on the type of sample (see below). The figures are based on replicate analyses of one blend and may differ from blend to blend, but this error should be less than $\pm 20\%$ in most cases.

A sample of frozen zooplankters taken in Baffin Bay on July 29, 1965, was kindly supplied by Dr W. Templeman, Fisheries Research Board of Canada, St. John's, Nfld. This sample was considered to be largely *L. helicina* with a few *L. retroversa* and was analysed as described above. In addition the presence of DMPT was confirmed by chromatographic procedures (Tocher et al., 1966).

Cooking Experiments

Fillets from fish 2A, 2B, 5A, 6B, 7A, and 9A were used in cooking experiments for taste panel assessment of product quality (Dyer et al., 1964). Particular samples were either baked at 260 C for 15 min or boiled in a closed polyethylene bag for 15 min. Appropriate controls of good quality fresh or frozen cod were used.

Freeze-Drying

Fillets from fish 5B and 7B were used. The fillets had been frozen in an irregular, rolled-up form. Accordingly, slices 7 mm thick were cut through center sections of the frozen tissue by band saw. These were freeze-dried in a Stokes (F. J. Stokes Corp., Philadelphia, U.S.A.) Model 21 weigh-type freeze-dryer. The average pressure range was 350–170 μ. Surface temperatures did not exceed the maximum given in Table III and were a few degrees lower for most of the drying period.

Table III. DMPT contents of freeze-dried cod fillets.

Sample No.	Freeze-drying conditions		DMPT content (*mg/g*)		Per cent recovery
	Max temp (*C*)	Time (*hr*)	Dried[a]	Original[b]	
5B	38	6.2	0.011	0.040	28
5B	82	5.5	0.0053	0.040	13
7B	82	5.5	0.015	0.090	16

[a]On wet basis, with net weight losses during drying of 79.4% (38 C) and 80.4% (82 C).
[b]Determined after 4 months at –20 C.

Satisfactorily reproducible analyses could only be obtained when the freeze-dried tissue was blended with water (1:20), filtered, and analyses carried out on the filtrate. Samples of flesh from the filter contained only traces of DMPT, approximately in proportion to the fluid content. The whole blend aliquots gave markedly less reproducible results than blends of fresh or frozen cod flesh, and representative samples taken from mixed chopped-up dried tissue from fish 5B (0.5 g dry weight plus 6.5 ml water) gave DMPT values ranging from 0.05 mg/g to 0.003 mg/g wet weight basis (*cf.* Table III). The dust from the polyethylene bag in which the dried tissue had been stored contained little or no DMPT.

RESULTS AND DISCUSSION

DMPT Deposition in Cod

At no time in the experiments was free DMS found in more than trace amounts in the flesh, viscera, or blood of the fish. The odour of fresh haddock includes DMS among other components, although the level is probably low (Mangan, 1959). Motohiro (1962) found that the digestive juices of chum salmon did not break down DMPT to give DMS. The *L. helicina* sample analysed in this work arrived packed with solid carbon dioxide, yet the DMS odour pervaded the whole room when the shipping container was opened. Analysis of the frozen *L. helicina* gave 0.02 mg/g DMS, which may represent as little as 10% of that originally present. DMS found in uncooked fish products is presumably produced in zooplankters and other plankton feeders such as oysters (Ronald and Thompson, 1964) by enzymes naturally present in the ingested phytoplankters originally containing DMPT. The death of the phytoplankter cells, if the species involved are similar in behaviour to *Polysiphonia fastigiata* (*lanosa*) on removal from water (Challenger, 1959; Cantoni and Anderson, 1956), would lead to breakdown of the DMPT with liberation of DMS until the digestive juices of the zooplankter stopped this action.

A dynamic balance must exist in the fish with respect to DMPT levels. Since DMPT was found in feces (Table I, fish 5B) absorption through the stomach or intestinal walls was not necessarily complete. The DMPT taken up by the bloodstream was probably in equilibrium with DMPT in various tissues and organs. It is not known if deposition occurred selectively in different parts of the muscle (see freeze-drying results). Collection of "drip" on thawing some frozen fillets gave DMPT levels (milligrams/millilitre) equal to or higher than those for the frozen samples. Although this point was not investigated further it suggests that the DMPT was present in the intercellular fluids. The level of DMPT in the blood is probably continuously lowered by removal either in the kidneys or through the gills, or both. The rate of removal in the liver would depend on suitable acceptors, assuming that the DMPT in the liver acts only as a methyl donor.

In any comparison between fish the relative sizes of fish and state of digestion, as well as DMPT consumed, are factors in the level of DMPT

found in the tissue. Metabolic rates are affected by water temperatures in addition to normal differences between individuals. Comparison of fish 2A and 2B with fish 3A and 3B shows that there was a rough relationship between level of dietary intake of DMPT and amount deposited. Fish 4A and 4B were killed 12 days after the last DMPT feeding in comparison with 2 days for fish 3A and 3B and had eaten nearly the same total of DMPT. The DMPT levels indicate that removal of the DMPT from the fish was rapid. Hence, if DMPT is deposited in cod flesh from a specific zooplankter diet, the DMPT content of the flesh would decline rapidly as the proportion of the particular zooplankter in the diet declined.

In one set of experiments DMPT was found in the livers (Table II, fish 2A–3B) in amounts roughly related to the level in the flesh and feeding rate, and in only small amounts in fish 4A–4B which had not been fed DMPT for 12 days. This result was not unexpected since Motohiro (1962) reported DMPT in chum salmon livers with proportions correlating over the fishing season with the degree of the DMS problem in canned salmon flesh. In a subsequent feeding experiment (fish 5A–7B), where analysis of the samples was delayed 4 months, the DMPT found in the livers was negligible. Some samples of a further group (fish 8A–9B) of livers had been placed in storage and one fish (9A) indicated that DMPT might be destroyed during frozen storage. Suitable transmethylation enzymes are known in certain mammalian visceral organs (Dubnoff and Borsook, 1948) and in livers of chum and sockeye salmon (Motohiro, 1962), although these are apparently not found in muscle tissue. Phospholipases in marine fish flesh remain active in frozen storage (Bligh, 1961) and the liver transmethylase involved in DMPT breakdown may also function under these conditions. A number of the livers in the various experiments were partly greenish in colour, suggesting the possibility of impairment of liver function in fish eating large amounts of DMPT. In frozen chum salmon livers Motohiro (1962) found a level of about 0.007 mg/g DMPT, but gives no comparable data for the salmon flesh. He observed that transmethylase activity in thawed liver preparations was twice as high in sockeye salmon liver as in chum salmon liver. This information suggests that cod livers may function at a greatly different rate from salmon livers, and also that the transmethylase is not deactivated by freezing.

Motohiro (1962) reported that *L. helicina* collected in the Pacific had approximately 0.02 mg/g DMPT, while the stomach contents of chum salmon (chiefly *L. helicina*) gave half this value. These values would of course be subject to seasonal and annual fluctuations depending on density and types of phytoplankters originally responsible for the DMPT (Ackman et al., 1965), density of the zooplankters in general, and the proportion of *L. helicina* in the salmon diet. This assumes that this organism feeds on a specific phytoplankter or phytoplankters, or may have a metabolic system which accumulates DMPT. Studies on oysters have shown that DMPT is accumulated in the viscera and is not rapidly removed, as in cod, when the intake of DMPT ceases (Ackman and Hingley, unpublished). The *L. helicina* from Baffin Bay

had, in addition to DMS, a DMPT content of 0.2–0.5 mg/g. These values were obtained on a frozen specimen mixture which included some shell fragments but not necessarily the full weight of shells, and may, therefore, be slightly high when compared with intact organisms as ingested by cod. The cod taken off Labrador in the blackberry season are described as particularly lean and gorged with zooplankton. A voraciously feeding fish such as 6A ate six herring chunks at one feeding. On this basis a diet exclusively of *L. helicina* could result in ingestion of more than 10 mg/day DMPT. Table I suggests that approximately 10 mg/day DMPT is the minimum intake necessary to deposit a significant level of DMPT in cod flesh. However, levels of DMPT in Labrador cod flesh when blackberry is prevalent remain to be determined.

Cooking

DMPT in the cod fillets tested (footnote e, Table I) produced no adverse effect upon baking. This material is thermally unstable (Challenger, 1959; Motohiro, 1962) and at the oven temperature involved (260 C) breakdown would probably be complete. The amount of DMS liberated in open baking (as distinct from cooking during canning; Motohiro, 1962) would be too small to be obvious, and the associated acrylic or propionic acids liberated would probably also be innocuous under these conditions.

Fillets from certain fish were sealed in polyethylene bags and immersed in boiling water for 15 min. Fillets from fish 2A, 2B, and 9A had been frozen for a few days prior to testing. A 2-cc sample of the gas from the bag of 2A was withdrawn by syringe and injected in a gas chromatograph. A large DMS peak was observed. On opening these bags the odour was considered objectionable. Although the texture was normal the taste panel spontaneously reported flavours reminiscent of turnip, parsnip, and radish with a lingering aftertaste. Fillets from fish 5A, 6B, and 7A were processed similarly on a separate occasion after 4 months frozen storage. Fillet 5A was known to contain little or no DMPT but the panel reported no off-flavour or aftertaste from fillets 6B and 7A.

The taste and aftertaste in these experiments may have arisen from a residue of *undecomposed* DMPT, since a similar methyl sulfonium compound (3-amino-3-carboxypropyldimethyl sulfonium salt) has been isolated from cabbage, turnip, pepper, and onion, among other vegetables (McRorie et al., 1954). This latter compound may contribute to the strong taste of these vegetables and also give DMS on cooking, although other sulfur compounds are also present (Ballance, 1961; Challenger, 1959). With fish the effect of DMPT on taste could be enhanced by heat, since the samples were moderately hot when presented to the taste panel, and also by other taste and flavour factors (Casey et al., 1965; Lea, 1963). DMS in the polyethylene bag does not necessarily imply, in the absence of controlled experiments, that all of this material was produced from DMPT, since DMS is well established as a volatile constituent of cooked fish (Hughes, 1964) and vegetables (Casey et al., 1963). Methionine is the usual source in vegetables, but the herring studied by Hughes

may have contained traces of DMPT in addition to other precursors. Alternatively, acrylic and propionic acids may have contributed to the strong flavours, but this is less likely since they should have been present in fillets 6B and 7A. The thermal decomposition of DMPT is profoundly influenced by *p*H (Motohiro, 1962), occurring more readily under alkaline conditions, or even at *p*H 7.0. Fillets 6B and 7A, and 5B and 7B (used in freeze-drying), were stored at −20 C for 4 months. The destruction of DMPT under mild thermal conditions (38 C) in freeze-drying suggests that prolonged frozen storage of cod flesh may give conditions predisposing DMPT to thermal decomposition; this may explain the negative results with 6B and 7A.

Hughes (1964) observed relatively large amounts of methyl mercaptan in cooked herring. Motohiro (1962) found methyl mercaptan levels to be higher in canned chum salmon having the petroleum odour than in canned sockeye or pink salmon. Since this thiol is probably derived from methionine (Ballance, 1961), any excess methionine produced by the action of DMPT as a methyl donor to homocysteine (Motohiro, 1962; Challenger, 1959) could also produce an objectionable volatile flavour in the form of the thiol on cooking in a closed container.

Freeze-Drying

Freeze-drying has been suggested as a means of processing which would remove the very volatile DMS should other means of handling blackberry cod not prove satisfactory. Table III indicates that this process would substantially reduce the DMPT level. The effect of drying temperature on the thermally unstable DMPT is noticeable in the results. For analyses carried out directly on the dried flesh, the highly erratic results may have been due to different textures and permeabilities of sample, since on blending a less homogeneous blend was obtained than with nondried tissue. The DMPT was, however, readily extracted on blending and it is reasonable to assume that localized concentrations of DMPT were formed within the flesh sample either in freezing or freeze-drying. The dust in the storage bag should have come chiefly from the outer surfaces where DMPT was removed by these processes or destroyed by local heating during the freeze-drying. Concentrations of DMPT within fish flesh could have very adverse effects on taste unless destroyed or dispersed on cooking.

ACKNOWLEDGMENTS

The authors wish to thank D. I. Fraser for arranging the cooking and taste panel experiments, and A. L. Wood for freeze-drying the fillets. The advice of W. J. Dyer was most helpful.

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Diel Activity and Vertical Distribution of Yellow Perch (*Perca flavescens*) Under the Ice[1]

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ABSTRACT

Use of echo sounders showed that, under the ice in Lake Mendota, yellow perch (*Perca flavescens* Mitchill) have a bimodal diel pattern of activity. The first peak of activity, during midmorning, was much higher than the second, in midafternoon. Schools of fish in mid water made up the bulk of the fish traces per hour although individual fish were also commonly recorded. At night, when activity was much reduced, principally single fish were observed. The fish swam much more slowly at night than during the day.

The activity pattern generally agreed with those reported by investigators for other lakes in that the perch were active during the day and relatively inactive at night.

No well-defined change in depth distribution was noted. There was a tendency for the fish to move away from the bottom to a limited extent at night but no marked movement was recorded. The majority of fish were most often recorded at a depth of 30–60 ft (9–18 m), the main concentrations being in areas of the lake more than 50 ft (15 m) deep.

INTRODUCTION

There are many reports in the literature on diel rhythms in the animal kingdom. Cloudsley-Thompson (1961) and Harker (1964) have written excellent reviews on the subject. Many of the studies dealing with freshwater fishes have confirmed that these animals have a daily rhythm of activity during the summer. Almost totally lacking, however, is information on daily movements of fishes in temperate lakes during winter when frozen surfaces and low temperatures make investigation difficult.

Previous investigators (Aitken, 1937; Hasler, 1945; Greenbank, 1956) have used nets set under the ice to obtain information on the movements of fishes. In the present study echo sounders were used to obtain information on the daily movements of yellow perch, *Perca flavescens* (Mitchill), in Lake Mendota, Wisconsin, during winter. We assumed that during periods of high activity more fish would swim beneath the transducer and be recorded than during periods of low activity. The number of fish recorded per hour was therefore used as an index of activity.

The use of echo sounders provides several advantages, the most important being the ease of placing a sounder in position in comparison with setting gill nets under the ice. In addition, the sounder gives information on the vertical distribution of the fish, their behavior, the size and shape of fish schools, and their relative rates of movement.

[1]Received for publication July 27, 1965.

J. Fish. Res. Bd. Canada, 23(4), 1966.
Printed in Canada.

METHODS AND MATERIALS

The study was conducted on Lake Mendota, a highly productive, eutrophic lake with winter characteristics very similar to other eutrophic lakes of temperate regions. The temperature in winter seldom varies more than two or three degrees from surface to bottom and oxygen is present throughout the depths until late March, when the values often become zero in the deepest waters. Light is often measurable to the bottom (as much as 10.8 lux in some years), intensity being governed by the thickness of the ice, the amount of snow cover, and the turbidity of the water. The ice reaches a thickness of at least 20 inches (51 cm) and the lake remains ice-covered for an average of 114 days. The mean closing date is December 19 while that of opening is April 6.

Recording

The data reported were obtained during the winters of 1960–65 by the use of two and sometimes three Bendix DR-9 depth recorders. The machines operate on DC (12 v), at a frequency of 50 kc/sec and with a pulse rate of 178/min. The traces are recorded on dry recording paper scaled from 0 to 200 ft (0–61 m) in depth graduations of 2 ft. The paper is advanced at 0.33 inch (0.8 cm)/min, and one roll has enough paper for approximately 43 hr of continuous recording.

Power was provided to the recorders by heavy-duty batteries (12 v, 205 amp-hr capacity) which supplied sufficient power for 24–35 hr of continuous recording, depending upon the temperature. The equipment was transported from site to site on the lake in an open vehicle, and, in 1965, in an airboat during snow-free periods.

Calibration tests using small perch and metal acoustical targets (9 inches^2, 58 cm^2) revealed that the sound from the transducer is transmitted through the water in the shape of a cone which, at a depth of 15 ft (5 m), becomes a cylinder with a diameter of 18–20 ft (6 m). The diameter remains practically constant to the maximum depths encountered in Lake Mendota (ca. 80 ft, 25 m). These tests were conducted with the echo sounder operating at full gain (setting #10).

Several stations (Fig. 1) were established early in the study. All were marked with signs and their locations determined by sextant. The stations ranged from 32 to 74 ft (10–23 m) in depth. After 1962–63, all recording was discontinued at the two stations shallower than 40 ft (12 m) since the numbers of fish recorded at these stations did not justify their maintenance. The results from the winters of 1960–63 indicated that most of the fish were concentrated in areas of the lake where the depth exceeded 50 ft (15 m).

Data were collected by continuous operation of the sounders for periods of 24 hr or longer at each station. The transducer was lowered, through a hole drilled in the ice, to a depth of 1–2 ft (0.5 m) below the ice and kept there for the duration of the recording period. After 1961, all records were made with

FIG. 1. Sites of the recording stations. The stations ranged in depth from 32 ft (10 m) at Station 2 to 74 ft (23 m) at the Basin Station.

the sounders set at maximum gain (#10). Experience from the preceding two winters indicated that the gain-settings #8 and 9 were too low to record fish swimming near the bottom in the deepest water.

INTERPRETATION OF TRACES

As yet there is no definite method for determining what species of fish or other organism is responsible for the trace appearing on the recording paper. A knowledge of the life history and behavior of the fish is essential for interpretation of the record. In Lake Mendota our problem was to distinguish yellow perch, the most abundant species in the lake, from white bass, *Roccus chrysops* (Rafinesque). Both are pelagic, schooling species although the schooling habit is not strictly adhered to. Confirmation of the species recorded is considered below.

An important aspect of how the traces are recorded is that the actual resolution of the machine is much less than the theoretical resolution, which is governed by the electrical characteristics. By resolution we mean the ability of the instrument to record separately two objects which are close together. The actual resolution is about 1.5–2.0 ft (45–60 cm), for 200 ft (61 m) of depth are compressed on recording paper only 7 inches (18 cm) wide. An observed resolution of 1.5–2.0 ft means that fish have to be separated by travel times of at least this magnitude to be recorded as separate individuals. Traces from fish closer together than 1.5–2.0 ft merge and appear as one large trace.

As some traces which appeared to be from individual fish may actually have been from more than one fish, the traces were assessed in this manner: the width of the trace of an individual perch (8 inches, 20 cm) was determined as roughly equivalent to 1.75 ft (50 cm) on the recording paper scale. The number

of fish represented by traces wider than 1.75 ft was taken as the number of times a single fish width was contained in the trace. This interpretation is biased in that the number of fish in each trace is the minimum number and therefore all estimates from traces wider than 1.75 ft are underestimates. Usually such a problem exists only when the number of fish in a school is determined. For the most part, however, interpretation of the traces was straightforward.

Most of the evidence on confirmation of the species recorded was obtained by fishing with gill nets. During the 1964–65 winter, nylon gill nets dyed red were set under the ice at intervals as close to 1 week as weather permitted. Four of the five nets used were 100 ft long × 6 ft deep with the following mesh sizes in inches (stretch measure): 1.5, 2.0, 2.5, and 3.5. The fifth net was 40 ft × 15 ft × 2.5 inches. A continuous recording of 24 hr was made at the netting site on the day before the nets were to be set. Examination of the recording paper on the spot showed the depth intervals where the fish were located. The usual set consisted of four nets placed under the ice and suspended so that they were fishing these intervals. The nets were fished for 24 hr and then removed. The numbers and kinds of fishes occurring in the different intervals were then recorded.

Of the other methods of confirmation tried, a remotely controlled underwater camera and an electric net each gave limited success. Some of the fish that were recorded on the sounder were simultaneously caught with hook and line. Also, we identified one school of fish visually as it passed beneath the transducer.

Vertical Distribution

The vertical distribution of the fish was determined mainly by counting the individual traces for the first 20 ft (6 m) and intervals of 10 ft below this depth. The day was divided into four periods: sunrise, 0430–0730 hr; day, 0730–1430 hr; sunset, 1430–1730 hr; and night, 1730–0430 hr. The data were recorded as the percentage of the total number of traces for that period occurring in each interval.

RESULTS AND DISCUSSION

Activity

Activity was generally high during daylight and low at night (Fig. 2). The typical cycle at all stations was as follows: At night the recording paper was characterized by traces that were extremely elongate (Fig. 3). Some traces persisted for more than an hour. These traces indicated that fish entered the sound field of the transducer and remained motionless or traversed the field very slowly. The fish were, most probably, in the state resembling sleep as reported by Hasler and Villemonte (1953). After several minutes the fish either drifted or swam out of the sound field.

The pattern of the traces changed abruptly as dawn approached. Traces became shorter as the fish began to swim more rapidly. The number of fish

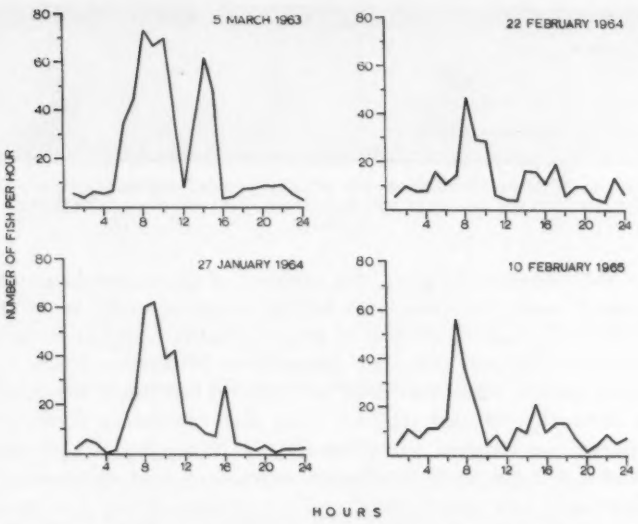

FIG. 2. Common pattern of diel activity. Data from four 24-hr recordings in 3 different years.

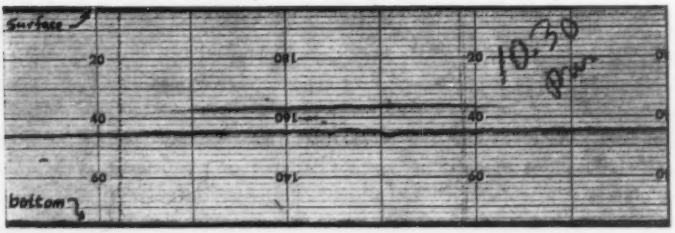

FIG. 3. Traces of two individual fish recorded during darkness. Depth of water 74 ft (23 m). Time 2227 hr to 2248 hr.

recorded per hour began to increase from 0500 to 0600 hr and then increased rapidly to a midmorning peak of activity between 0700 and 1100 hr. Part of the increase in activity was due to fish which were thought to be feeding along the bottom. Very likely these fish were foraging for *Chironomus* larve, which form a part of their winter diet (Hasler, 1945). The increase in numbers of fish presumably feeding along the bottom paralleled the increased activity in mid water. The appearance of schools of fish in mid water, however, was largely responsible for the peak period of activity during mid morning. The schools of fish varied in size, but often the distance from the top of the school to the bottom was 30–40 ft (9–12 m) (Fig. 4). The schools caused traces typically spindle-shaped or bell-shaped and, during the morning hours, moved rapidly through the area of sensitivity.

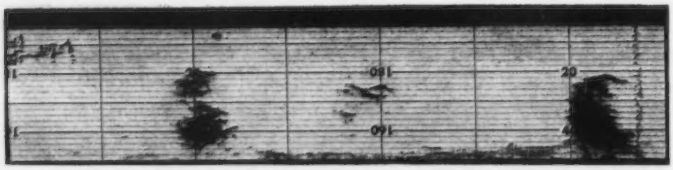

FIG. 4. Typical traces from fish schools recorded during daylight. The distance from the top of the schools to the bottom was often more than 30 ft (9 m).

After the midmorning peak, the number of traces per hour was usually lowest around noon. However, this lull appeared as early as 1100 hr or as late as 1400 hr. A midday decline in activity was also reported for immature black bullheads, *Ictalurus melas*, by Darnell and Meierotto (1965).

Activity usually again increased to a second but much lower peak in the afternoon between 1300 and 1600 hr. After this, movement declined until the nocturnal level was reached, sometime after 1700 hr. Activity fluctuated during the night, but the next significant increase in movement occurred with the next sunrise.

Schools of fish were seldom recorded later than 1730 or 1800 hr; during the night, principally single fish, if any, were observed. This agrees with the evidence that schooling is mediated through the eye and depends upon the ability of members of the school to see one another (John and Haut, 1964). Occasionally at night, traces appeared on our recording paper that resembled a loose aggregation of fish and, in these instances, the individual fish were often 4–6 ft (1–2 m) or more apart. Perhaps this kind of aggregation results when darkness envelops a school of fish and its members lose visual contact with each other and slowly drift apart. Alabaster and Robertson (1961) observed that schools of European perch (*Perca fluviatilis*) held in the laboratory also break up in the evening when light becomes reduced.

There was considerable variation in the magnitude of activity near each of the two daily peaks and this may have been due in part to two factors: the echo sounder surveyed an extremely small part of the lake, and our use of clock rather than solar time may not have been realistic for the activity cycle of the perch.

Cloudsley-Thompson (1964), in his study of the Nile crocodile, observed that periodicity often becomes more apparent when the results of a few days observations are averaged. Figure 5 compares the results when the records of two different winters are averaged. The close agreement of various points on the activity curves for the two winters is evident.

The activity pattern described for the yellow perch in Lake Mendota generally agrees with the results of studies from different lakes by other investigators in that perch are active during the day and relatively inactive at night. The times at which maximum activity occurs, however, seem to be different from one lake to another. Brown and Rosen (1951) reported that perch in Lake Okoboji are most active from 1000 until 1400 hr and again from

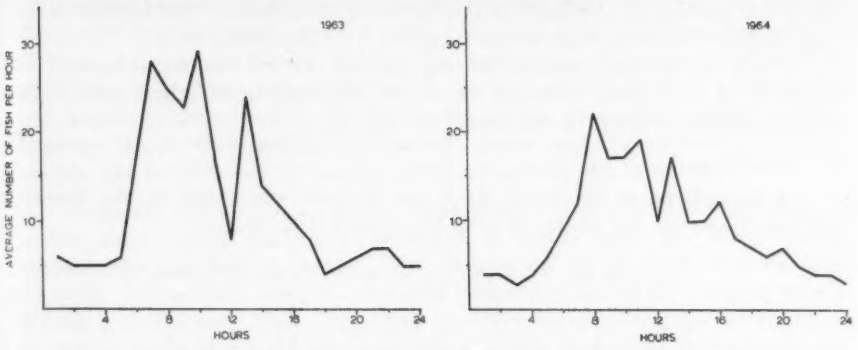

FIG. 5. Mean numbers of fish recorded per hour for each of two entire winters.

1600 to 1800 hr. Carlander and Cleary (1949) stated that the perch they studied appeared to be most active during the afternoon. Sieh and Parsons (1950) found that perch activity in Clear Lake, Iowa, was most intense from 1600 to 1800 hr while Scott (1955) caught the greatest number of perch in his nets at 1700 hr with the fish becoming inactive after sunset.

FACTORS INITIATING FISH MOVEMENT

No attempt was made during this study to measure routinely environmental parameters which could be related to fish movement. Incidental evidence, however, suggested that light might have been one of the factors initiating fish movement at dawn. We noted a general tendency for the fish to become active at successively earlier times in the morning as the winter seasons progressed. This suggested that the onset of fish movement was geared to the sun which, as the winter progressed, rose at successively earlier times.

DEPTH DISTRIBUTION

DAILY

No well-defined, periodic change in the depth distribution of perch was noted during this study; the perch in Lake Mendota do not make a daily vertical migration in winter. A majority of the fish were found most often within the depth intervals of 30–60 ft (9–18 m). In some years (e.g. 1965) more of the fish occurred in the upper part (30–40 ft, 9–12 m) of this interval than in the lower (50–60 ft, 15–18 m) while in others (1961), the reverse was true. The daily distribution was generally skewed with most of the fish being recorded in the lower half of the water column. At night a larger proportion of the fish occupied the upper half of the water column than during the day but the majority were still found in the lower parts of it. Fish were seldom recorded along the bottom at night, most of them being at least 4 ft (1 m) above it.

With the coming of daylight fish were again recorded on the bottom when they began to exhibit what we have termed "feeding behavior."

Many of the fish recorded during the day within the depth interval of 10–30 ft (3–9 m) were members of schools. An individual school may cover a large vertical interval as was shown in Fig. 4.

SEASONAL

A movement of fish away from the bottom was noted at the deepest station (Basin) as the winter of 1963 progressed (Fig. 6).

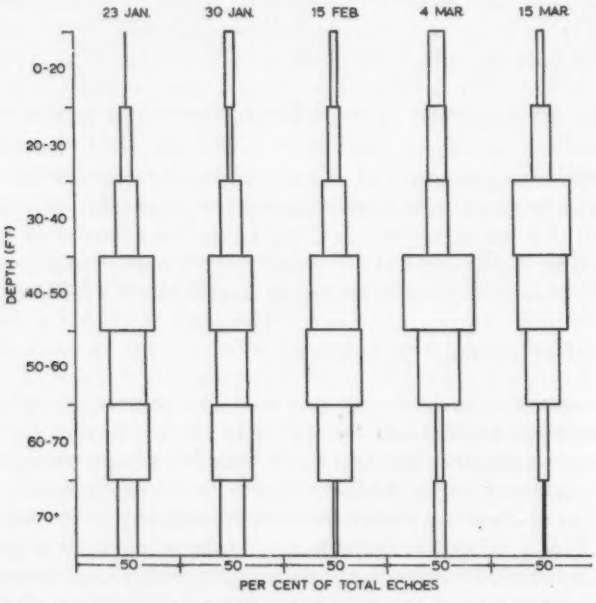

FIG. 6. Movement of fish away from the bottom at the Basin station as the 1963 winter progressed. The width of the block for each depth interval represents the per cent of the total number of fish which were recorded in that interval during a 24-hr period.

The shift upward was perhaps due to a reduced concentration of dissolved oxygen in the lowermost part of the water column; in many winters no oxygen could be detected in the deepest waters in March (Stewart, MS, 1965). Since we did not measure dissolved O_2, we can only speculate that inadequate oxygen was the factor responsible for the observed distribution of fish.

The factors which control the depth at which fish are found from one day to the next are unknown. Northcote et al. (1964) presented evidence that the peamouth chub (*Mylocheilus caurinus* Richardson) roughly followed the 1 lux level of illumination in Nicolet Lake, B.C., during the summer. Under ice cover, however, the peamouth chub did not exhibit a diel vertical migration and remained near the bottom at all times. Balls (1951) suggested that

herring are negatively phototactic during the day and remain on the bottom. They only begin to move upward when light is of sufficiently low intensity for them to be "comfortable." Light may be important in regulating the depth at which perch are found in Lake Mendota during winter, but, if so, at extremely low levels of illumination. In our study, there seemed to be no relation between the depth at which the fish were found and the amount of snow on the ice, the turbidity of the water, or the amount of sky overcast.

Ferguson (1958), in laboratory experiments, found that temperature, if acting alone, could determine the depth at which perch are found during summer. In the field, however, this response to temperature was modified by other factors such as light and social interaction of the fish, among others. Horak and Tanner (1964), concluded that the observed vertical distribution of yellow perch in a Colorado reservoir was independent of the temperature regime to which the fish were exposed. Since the winter temperature of Lake Mendota seldom varies more than 2 or 3 degrees from top to bottom, it probably is not important in regulating the vertical distribution of the fish.

Confirmation of Species Recorded

Throughout this article we have referred to the traces which appeared on our recording paper as being those from yellow perch while, in fact, some of them may have been from white bass. It will be recalled that the major part of the activity curve during the day was owing to fish which were found in schools. If we could identify the schools to species, we could account for a major part of the activity cycle. Both white bass and perch are schooling species in summer, although not strictly so since solitary individuals of both species are observed by divers.

Hasler (1945) presented evidence that yellow perch continue this schooling habit throughout the winter. In several instances during the winter of 1965, clusters of perch were so arranged in the gill nets as to suggest that they perhaps were schooling when captured. White bass, on the other hand, were always well separated from each other and gave no indication at all that they had been schooling. Accordingly, we cannot say whether the schooling habit of the white bass continues into and throughout the winter.

We observed many times during the various winters of the study that when ice fishermen caught perch, several, all more or less the same size, were caught in succession. No fish were caught for a time, after which several additional perch were taken, suggesting that perhaps schools of perch were moving through the area. On one occasion during the winter of 1965, we were fortunate to visually observe a school of perch as they passed at a depth of 18–24 ft (5–7 m) beneath the transducer and were recorded (Fig. 7). The school consisted of from 6 to 10 individuals and the light conditions were such that the vertical markings on the fish stood out very prominently so that there was no doubt as to their identity.

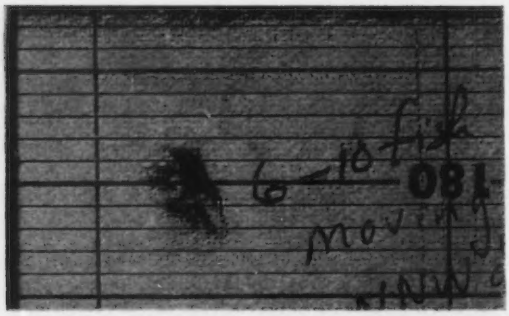

FIG. 7. A school of yellow perch (6–10 fish) that was visually observed as it passed at a depth of 18–24 ft (5–7 m) beneath the transducer.

On three or four occasions, individual fish appearing near the bottom on the recording paper were captured with hook and line. In every case, the fish was a yellow perch.

The results of the winter netting of 1965 suggested that most of the fish recorded on the echo sounder were perch. A series of 10 sets, each of 24 hr duration, made throughout the winter and early spring, captured 427 perch and 74 white bass, a ratio of 6:1. Tibbles (MS, 1956), captured 7296 perch and 421 white bass, a ratio of 17:1, in an intensive gillnetting operation on Lake Mendota from mid July 1954 to April 1955. In addition, during the winter of 1956, a creel census conducted on Lake Mendota revealed that ice fishermen caught nearly 1.5 million perch during the short season of 8–10 weeks. The perch population must be extremely large if such a number can be removed by the relatively inefficient method of hook and line fishing. White bass, on the other hand, are seldom taken by winter anglers even though minnows, a popular winter bait, are used with much success during the summer.

On the basis of the present evidence, we feel certain that the major portion of the fish recorded on the echo sounder are perch and that the activity cycle we have described is a true reflection of the movements of yellow perch in winter in Lake Mendota.

ACKNOWLEDGMENTS

The authors would like to thank the members of the Laboratory of Limnology, especially Clyde W. Voigtlander, for assistance with the field work. Part of the data utilized in this report was accumulated by Roderick C. Stone while he was a research assistant in this laboratory.

The investigations reported here were supported in part by grants from the Wisconsin Conservation Department, the Office of Naval Research [Nonr-1202 (04), NR301-903], and the National Science Foundation (G-9679).

The manuscript was completed while G. L. Hergenrader held an Educational Fellowship from the U.S. Bureau of Commercial Fisheries.

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Appropriate Size Limits for Lemon Sole (*Parophrys vetulus*) in the Strait of Georgia[1]

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ABSTRACT

Estimates of the appropriate size limit for four separate stocks of lemon sole (*Parophrys vetulus*) in the Strait of Georgia are obtained using the method of Allen (1953). The calculated sizes for optimum yield agree in general with those calculated for the same stocks by Forrester and Ketchen (1963) using methods of Ricker (1958). A preliminary estimate of the additional yield which would be achieved by increasing the minimum size utilized in the only stock in which a further increase is required suggests that it would be about 4%.

INTRODUCTION

The lemon sole is one of the principal species taken by the trawl fishery in the lower Strait of Georgia between Vancouver Island and the mainland of Canada, forming about one-quarter by weight, and more by value, of the total quantity of food fish caught in the area. The main features of the fishery have been reviewed by Forrester and Ketchen (1963), who examined the question of the effect on the catch of varying the age of entry to the fishery using analytical methods based on those of Ricker (1958). They concluded that, in general, the maximum yield would be obtained with an age at entry of 4 years, although this age should be varied locally to some extent depending on the intensity of fishing in the different areas within the Strait. This age corresponds to a length of 30 cm, or to a mesh size of $4\frac{1}{2}$ inches (Forrester and Ketchen, 1963). This size agrees closely with the minimum marketable size (12 inches) which has been in force for many years, and with the legal mesh size in effect since late 1962. Prior to this date a mesh size of about $3\frac{1}{2}$ inches was commonly in use.

An alternative approach to the problem of estimating the appropriate size limit is provided by the fact that, when the limit is correctly set to produce the maximum weight of catch,

$$W_c = E'\overline{W}$$

where W_c = weight of typical fish at the limiting size

$\overline{W}$ = mean weight of the fish caught

[1]Received for publication November 3, 1965.
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J. Fish. Res. Bd. Canada, 23(4), 1966.
Printed in Canada.

E' = proportion of fish reaching W_c which are ultimately caught (Allen, 1953).

MATERIAL

For the lemon sole, data regarding the size distribution in four areas of the Strait of Georgia are available from samples of commercial catches over a number of years, generally between 1952 and 1961, and shown in detail for each area in Table I. During this period the size composition of the stocks showed some irregular fluctuations which were apparently due more to variations in recruitment than to the effect of fishing. Over the Strait as a whole the fishing effort has tended to decline although there has been variation between the different areas, the decline being most pronounced in the Gulf Islands area and indiscernible on the Cape Lazo grounds. In considering the correct size limit for a fish stock, it is necessary, except in times of rapid change of effort, to determine the value which will give the best results over a period of years during which there may be natural fluctuations in the composition of the stock. In analysing the data, therefore, the results have been pooled for each area by taking the average composition for the years for which data were available; and it has been assumed that these compositions developed in the presence of fishing intensities corresponding to the fishing mortality rates given for the areas by Forrester and Ketchen (1963).

Two further assumptions have also been made. The first is that the stocks in the four areas within the Strait are, for practical purposes, distinct. This agrees with the conclusion reached by Forrester and Ketchen (1963, p. 12), on the basis of tag returns, that there are three principal stocks in the Strait of Georgia region; one in the Gulf Islands area, one in the "Sandheads" area of the Fraser River estuary, and a third in Area 14; and that the last can be subdivided into a stock on the Cape Lazo grounds and one in Baynes Sound, which includes Union Bay, and that these mix only slightly. The second assumption is that the catches from which the samples were drawn are representative of the populations in the various areas. The influence of mesh and market selection is discussed at a later point in this paper, and it is considered that above about 12 inches total length the catches are representative of the stocks on the actual fishing grounds. There remains the question whether the stocks on the fishing grounds are fully representative of those in the areas from which they are drawn, or whether there is some differential distribution by age within the area occupied by each discrete stock. In the absence of satisfactory evidence to the contrary it is assumed that stocks on the fishing grounds are fully representative.

In estimating the average weight at each length, the relationship

$$W = .00034065L^{3.0074}$$

has been used, W being the weight in ounces and L the total length in centimeters. This relationship has been derived from a regression line fitted to data obtained from samples of fish taken from Union Bay in the period 1951–54.

TABLE I. Variation of $W_e/\overline{W}$ with size for the four areas.

Length (*cm*)	Weight (*oz*)	Gulf Islands 1954–60			Cape Lazo 1952–61			Union Bay 1953–60			Fraser River 1947–53		
		%	$\overline{W}$	$\frac{W_e}{\overline{W}}$	%	$\overline{W}$	$\frac{W_e}{\overline{W}}$	%	$\overline{W}$	$\frac{W_e}{\overline{W}}$	%	$\overline{W}$	$\frac{W_e}{\overline{W}}$
25	5.45	..	..	..	..	..	..	..	..	..	..	..	..
26	6.14	..	..	..	..	..	..	..	..	..	..	..	..
27	6.87	.3	16.55	0.415	0.2	16.87	0.407	..	..	..	0.6	13.11	0.524
28	7.67	1.6	16.60	0.462	0.6	16.89	0.454	0.2	18.34	0.418	3.1	13.13	0.584
29	8.52	2.8	16.73	0.509	1.5	16.93	0.503	0.8	18.40	0.463	7.2	13.31	0.640
30	9.43	5.4	16.99	0.555	4.3	17.08	0.552	2.0	18.49	0.510	11.9	13.70	0.688
31	10.41	8.2	17.43	0.597	7.8	17.43	0.597	4.6	18.65	0.558	12.8	14.35	0.725
32	11.45	11.2	18.14	0.631	9.9	18.05	0.634	6.9	19.08	0.600	14.8	15.14	0.756
33	12.56	10.6	19.20	0.654	11.6	18.94	0.663	8.4	19.68	0.638	11.8	16.24	0.773
34	13.74	10.0	20.38	0.674	10.0	20.08	0.684	9.6	20.44	0.672	10.9	17.39	0.790
35	15.00	7.9	21.70	0.691	9.6	21.24	0.706	11.0	21.39	0.701	7.9	18.89	0.794
36	16.32	6.7	22.98	0.710	8.5	22.60	0.722	11.1	22.66	0.720	4.8	20.50	0.796
37	17.72	5.8	24.27	0.730	6.5	24.07	0.736	8.6	24.20	0.732	3.6	21.90	0.809
38	19.20	5.0	25.53	0.752	5.5	25.49	0.753	7.7	25.73	0.746	2.5	23.30	0.824
39	20.77	3.9	26.83	0.774	4.5	26.93	0.771	6.0	27.47	0.756	2.2	24.57	0.845
40	22.41	3.6	28.01	0.800	3.7	28.36	0.790	4.5	29.17	0.768	1.7	25.99	0.862
41	24.13	3.2	29.17	0.827	2.8	29.75	0.811	3.4	30.81	0.783	1.3	27.45	0.879
42	25.94	3.8	30.37	0.854	2.7	30.95	0.838	2.6	32.34	0.802	0.7	28.95	0.896
43	27.85	2.7	32.08	0.868	2.4	32.30	0.862	2.7	33.63	0.828	1.0	29.91	0.931
44	29.85	2.3	33.65	0.887	2.2	33.65	0.887	2.0	35.24	0.847	0.6	31.62	0.944
45	31.92	1.8	35.46	0.900	2.3	35.11	0.909	2.1	36.60	0.872	0.3	33.38	0.956
46	34.12	1.2	37.49	0.910	1.3	37.24	0.916	1.7	38.29	0.891	0.2	34.88	0.978
47	36.40	0.7	39.65	0.918	0.8	39.18	0.929	1.3	40.00	0.910	0.1	36.40	1.000
48	38.75	0.6	41.53	0.933	0.7	40.91	0.947	1.2	41.71	0.929	..	..	..
49	41.25	0.2	44.35	0.930	0.2	43.42	0.950	0.7	43.92	0.939	..	..	..
50	43.84	0.2	45.85	0.956	0.3	44.50	0.985	0.5	46.00	0.953	..	..	..
51	46.53	0.1	47.91	0.971	0.1	46.53	1.000	0.2	48.67	0.956	..	..	..
52	49.32	0.1	49.32	1.000	..	..	..	0.1	50.79	0.971	..	..	..
53	52.24	..	..	..	..	..	..	0.1	52.24	1.000	..	..	..

OPTIMUM SIZE LIMIT

Table I shows the average size distribution and values of $\overline{W}$ and $W_c/\overline{W}$ for the samples from each of the four areas. The values of $\overline{W}$ were obtained by dividing the cumulative sum of the products of percentage and weight by the cumulative sum of the percentages, summation being of course begun at the maximum values. As calculated in this way $\overline{W}$ for any given length can be taken to be an estimate of the mean weight of fish in the catch if a size limit of that length were applied to fishing on the corresponding population. $W_c/\overline{W}$ therefore estimates what the proportion ultimately caught (E′) should be if the population were being exploited eumetrically. If an estimate of E′ is available, and this value is higher than the value of $W_c/\overline{W}$ for the minimum size in the sample, then an estimate of the correct size limit is that length at which $W_c/\overline{W} = E'$. It should be noted that this only applies if the estimate of E′ is correct and if the change in the size limit is not accompanied by any change in fishing intensity, and therefore in E′.

Estimates of E′ for the various areas are available from Forrester and Ketchen (1963, p. 55) since, if a constant ratio between natural and fishing mortality at all ages is assumed,

$$E' = \frac{F}{Z} \text{ in international units (Holt et al., 1959)}$$

$$= \frac{p}{i} \text{ (Forrester and Ketchen, 1963).}$$

E′ as used here should be distinguished from E in the international notation since it measures the probability of capture at any future time, and not within a fixed period, as does E. The average values of E′ for the four areas are therefore taken to be

Fraser River 0.67,

Gulf Islands 0.63,

Cape Lazo 0.51,

Union Bay 0.43.

Before relating these values of E′ to the tabulations of $W_c/\overline{W}$ it is necessary to examine further some characteristics of the size distribution data. As stated, the samples were obtained from commercial landings; they have, therefore, been subjected initially to mesh selection by a mesh of $3\frac{1}{2}$ inches. This would have a 50% selection point at about 23 cm (Forrester and Ketchen, 1963, p. 55), and probably produced a negligible reduction above 25 cm (Anon. 1957).

Unfortunately, the samples had also been subject to market selection by the fishermen prior to sampling. This resulted in the discarding of the great

majority of fish below 12 inches (about 30 cm), and of very few over this size. The effect of this form of selection cannot be determined as precisely as can that of mesh selection. It will be inherently liable to variation due to such factors as the characteristics of individual fishermen, and the state of the market. These could affect not only the size at the 50% selection point, but also the shape, including both symmetry and spread, of the selection ogive. Very extensive studies would therefore be necessary to obtain quantitatively useful data on the characteristics of this selection. This has not been attempted, and the only data available are a few comparisons between commercial landings and catches taken experimentally with a trawl with a fine-meshed liner, at roughly the same time and place. From these it appears that at sizes below 30 cm the proportion of discards has been so large and so uncertain that no useful conclusions can be drawn from this part of the size distribution, and that at this size, and for 2 or 3 cm size groups above it, the samples underestimate the proportions in the actual populations from which they were drawn.

When selection leading to the rejection of some of the smaller fish has been in operation, e.g. mesh or market selection, the proportion of any size group affected will be smaller in the sample than in the original population. The average size of all fish from and including this size upwards will therefore be greater for the sample than for the population, so that $\overline{W}$ is overestimated. W_c is of course not affected, and thus the ratio $W_c/\overline{W}$ is underestimated. Since $W_c/\overline{W}$ normally increases with W_c for any given population or sample, the value of W_c at which $W_c/\overline{W}$ equals any given E' is higher for the sample than for the population.

In Table I the values of $W_c/\overline{W}$ closest to the estimated values of E' are marked with an asterisk for each area. In all the areas except the Gulf Islands the lengths at which $E' = W_c/\overline{W}$ in the samples are less than 30 cm. The results will, therefore, have been affected by market selection and the actual lengths at which $W_c/\overline{W} = E'$ will, therefore, be less than those marked in the Table. Some indication of the probable magnitude of the errors can be obtained from examination of comparable data for commercial and complete catches. The best samples available are some taken in the Fraser River area in August 1962. These are set out in Table II. This shows that the appropriate value of $W_c/\overline{W}$ (0.67) occurs at 28 cm in the experimental sample, and between 28 and 29 cm in the commercial landings. At first sight the difference between the two samples is in the opposite direction to that expected. A clearer picture of the actual position can be obtained from a graphical analysis of the data as in Fig. 1. The difference in the upper part of the two curves where they should not be affected by market selection shows that the two samples were not in fact representative of the same population. It is noticeable, however, that at sizes below 30 cm the curve for the commercial sample declines much more rapidly than that for the experimental sample, and this is in accordance with expectation. The probable trend at the lower end of the commercial sample, if the effect of selection were removed, would be more nearly parallel to that of the experimental sample, as indicated by the dotted line. It appears,

TABLE II. Comparison of $W_c/\overline{W}$ for experimental and commercial samples from the Fraser River area.

		Experimental			Commercial		
Length (*cm*)	Weight (*oz*)	%	$\overline{W}$	$\frac{W_c}{\overline{W}}$	%	$\overline{W}$	$\frac{W_c}{\overline{W}}$
21	3.23	0.1	10.15	0.318	..	..	..
22	3.71	0.3	10.16	0.365	..	..	..
23	4.24	0.4	10.18	0.417	..	..	..
24	4.82	2.0	10.20	0.472	..	..	..
25	5.45	5.7	10.31	0.529	..	..	..
26	6.14	6.9	10.61	0.579	..	..	..
27	6.87	8.8	10.97	0.626	2.6	11.68	0.588
28	7.67	11.7	11.45	0.670	3.8	11.81	0.649
29	8.52	10.4	12.14	0.702	7.5	11.98	0.711
30	9.43	9.3	12.84	0.735	18.4	12.28	0.768
31	10.41	9.9	13.55	0.768	18.0	13.06	0.797
32	11.45	7.9	14.45	0.793	15.4	14.03	0.816
33	12.56	7.6	15.33	0.819	9.4	15.19	0.827
34	13.74	5.4	16.42	0.836	6.0	16.19	0.849
35	15.00	4.3	17.48	0.858	7.5	16.98	0.883
36	16.32	2.8	18.60	0.877	5.6	18.31	0.891
37	17.72	2.5	19.57	0.906	1.5	20.31	0.872
38	19.20	2.0	20.71	0.927	1.5	21.25	0.903
39	20.77	0.8	22.17	0.937	0.8	22.43	0.926
40	22.41	0.8	23.12	0.969	1.1	23.09	0.970
41	24.13	0.3	24.58	0.981	0.8	24.13	1.000
42	25.94	0.1	25.94	1.000	..	..	..

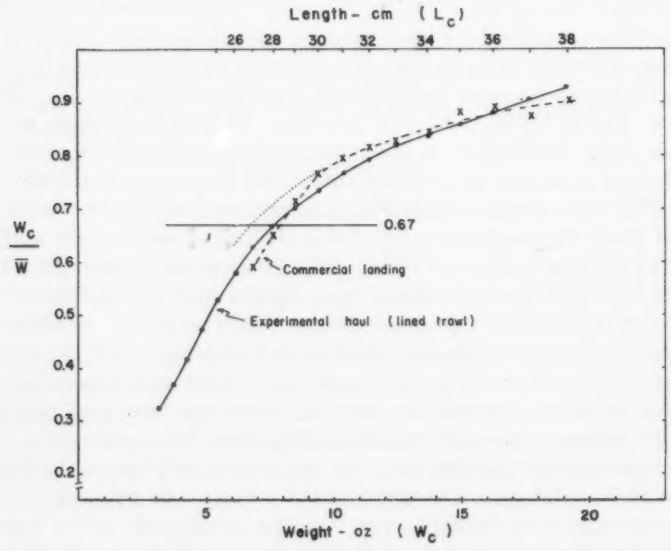

FIG. 1. Variation of $W_c/\overline{W}$ with size for commercial and experimental samples from the Fraser River area.

therefore, that in this case the error introduced by selection is of the order of 1.5 cm, and that the error will increase with the amount by which the length under consideration falls short of 30 cm. On this basis the approximate lengths at which $W_c/\overline{W}$ would equal E′ for the various areas are

Gulf Islands 32 cm,

Cape Lazo 28 cm,

Union Bay 27.5 cm,

Fraser River 28.5 cm.

Thus all these estimates of the optimum size at first capture are above that produced by the mesh size commonly in use during the period covered by the data, and that for the Gulf Islands is greater than that prevailing at the present time.

These conclusions do not depart greatly from those reached by Forrester and Ketchen (1963) that at Cape Lazo and Union Bay the maximum yield would be obtained with an age at first capture of $3\frac{1}{2}$ years, corresponding to a length of 28 cm, and that for the Gulf Islands and the Fraser River the maximum would be at 4 years or 30 cm.

CHANGES IN YIELD

Methods have recently been developed (Allen, in press) for estimating by rather similar techniques the approximate amount by which the yield would be increased if a size limit is adjusted from an incorrect value to one which will give the maximum yield at the same fishing intensity. Since the growth of this species is apparently asymptotic and follows a curve which fits a simple von Bertalanffy curve reasonably well, it is preferable to use the models based on the von Bertalanffy curve rather than those assuming exponential growth. Owing to the deficiencies of the data no useful estimates would be obtainable for situations involving actual or potential size limits less than 30 cm. It seems worthwhile, however, to examine in this way the increase which would be obtainable in the Gulf Islands area if the size limit were increased from 30 cm to 32 cm. Examination of the data suggests that in this area market selection had substantially reduced the proportion of fish recorded at 30 cm and had also affected those at 31 cm to a lesser extent. The percentages at these two lengths were, therefore, adjusted to 14.3 and 13.1, respectively, prior to any further calculations (the "percentage" total then of course exceeds 100 but this does not affect the results).

Following the method referred to, the ultimate new yield is the product of the immediate decrease resulting from the cutting out of a part of the catch, and an ultimate increase in the population above the new limit due to increased survival; i.e.

$$\frac{Y_2}{Y_1} = \frac{Y_k}{Y_1} \cdot \frac{Y_2}{Y_k}$$

where Y_1 is the present yield, Y_k the immediate new yield, and Y_2 the ultimate new yield.

Y_k/Y_1 can be easily estimated from the original data as the cumulative weight of the sample from the upper end down to the new limit divided by the cumulative weight down to the old limit. For the present sample, after adjustment, this equals 0.846.

Y_2/Y_k has been shown to equal (Allen, in press)

$$\left(\frac{L_\infty - L_1}{L_\infty - L_2}\right)^{\frac{Z}{K}E'}$$

where L_∞ and K are the parameters of the simple von Bertalanffy equation fitted for the part of the growth curve including the old and new limit sizes; Z is the present total mortality rate between these sizes; and L_1 and L_2 are the old and new limit lengths, respectively.

Forrester and Ketchen (1963) have provided a growth curve for the lemon sole in the Strait of Georgia, and a Ford-Walford plot of lengths at each age read off this curve gives an estimate of L_∞ of 43.5 cm.

It has also been shown (Allen, in press) that an estimate of Z/K can be obtained from a graph of logarithm of frequency against logarithm of length. When, as in the present case, the data have been grouped by equal length intervals

$$\frac{Z}{K} = 1 - G\left(\frac{L_\infty}{L} - 1\right)$$

where G is the slope of the line at length L and is negative. If $\left(-\frac{1}{G}\right)$ is plotted against $\left(\frac{1}{L}\right)$ a straight line fitted to the relevant points should intercept the $\left(\frac{1}{L}\right)$ axis at $\left(\frac{1}{L_\infty}\right)$, and should have a slope

$$\frac{L_\infty}{\frac{Z}{K} - 1}$$

Figure 2 is a log-log plot of frequency against length and Fig. 3 a plot of $\left(-\frac{1}{G}\right)$ against $\left(\frac{1}{L}\right)$ for the freehand curve drawn through the points of Fig. 2. A straight line has been drawn passing through the points for the lower lengths and intercepting the $\left(\frac{1}{L}\right)$ axis at 1/43.5. It will be seen that this line, as well as passing through the point corresponding to the value of L_∞ obtained from the growth curve, is also a reasonably good approximation to the slope of the points up to about 37 cm. Above this length the points depart from this line and have a trend which, if extended, would intercept the 1/L axis in the region corresponding to L_∞ = 61 cm. It is likely, however, that this divergence is

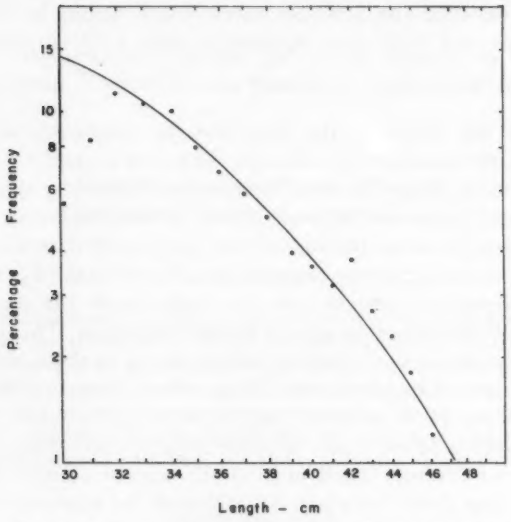

FIG. 2. Logarithmic plot of frequency against length for the Gulf Islands area.

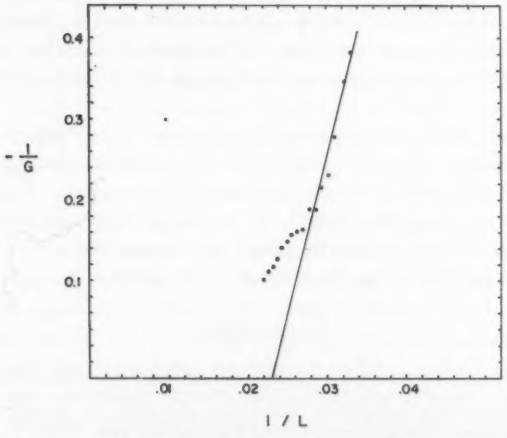

FIG. 3. Reciprocal of gradient plotted against reciprocal of length for the data in Fig. 2.

mainly due to the increasing effect of individual variation at the greater lengths, and that the line shown provides the most satisfactory basis for estimating Z/K at the stage in the life history with which we are concerned. From the slope of this line Z/K is estimated as 2.053. Therefore

$$\frac{Y_2}{Y_k} = \left(\frac{43.5 - 30.0}{43.5 - 32.0}\right)^{2.053 \times 0.63} = 1.230.$$

The ratio between the ultimate catch which would be obtained with a 32 cm size limit and that now obtainable with a 30 cm limit is therefore $\frac{Y_2}{Y_1} = 0.846 \times 1.230 = 1.040$.

In view of the nature of the data and the subjective methods used in fitting the lines, the errors of this estimate cannot be properly assessed although they could be fairly large. It must be stressed, however, that provided the original size distributions are representative of the population the increase in the limit must lead to some increase in the catch, and that the errors cannot, therefore, be large enough in the negative direction to make Y_2/Y_1 less than 1.0.

It is of interest to compare this conclusion with the results obtainable by application of the Beverton and Holt yield equation. This may be applied approximately in the present case by interpolating in the published tables of the yield function (Beverton and Holt, 1964). From $Z/K = 2.305$ and $E' = 0.63$ we have $M/K = 0.763$, and $c(= L/L_\infty) = 0.609$ for the 30 cm limit and 0.735 for a 32 cm limit. Interpolating in the tables the ratio of the yields for the two limits is found to be 0.985, approximately. As pointed out before, a ratio less than 1.0 must be incorrect. It appears, therefore, that this method is not applicable in the present instance, probably either because the mortality rates change with age, or because the growth curve is not sufficiently close to the simple von Bertalanffy model. The change in slope of the part of Fig. 3 for higher lengths does suggest that at least one of the parameters concerned changes with age, although it could also be influenced by the increasing effect of individual variation as the annual increments become less.

The method used earlier has less rigorous requirements since it only assumes exponential mortality and von Bertalanffy growth with constant parameters over the period of life between the two limits. It is unaffected by subsequent changes in either growth or mortality with age, and should, therefore, give a more reliable estimate, apart from sampling errors, of the relative increase in yield produced by the change in the limit size.

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Reproduction in *Sphyrion lumpi*, a Copepod Parasitic on Redfish (*Sebastes* spp.)[1]

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ABSTRACT

Simultaneous development of ova in ovaries and of embryos in eggsacs of *Sphyrion lumpi* suggested that more than one spawning of this parasite was probable in nature. The functional morphology of the trunk also suggested that spawning and eggsac formation occurred during a short period when the ova were ready to be extruded. Six arbitrary stages of maturity of females were defined. Only 27% of the adult females were ovigerous. Thirteen males were found on 12 of the 263 females examined. Fecundity estimated from numbers of eggs in eggsacs was 6–30 thousand eggs at trunk sizes of 30–45 mm.

INTRODUCTION

The condition of ovaries, sizes of ova, and stages of development of embryos in eggsacs of *Sphyrion lumpi* Krøyer suggest multiple spawning. This paper presents histological evidence for this and defines six arbitrary stages of maturity for females. Males were defined as immature or adult.

Maturity stages of *S. lumpi* have not been defined in the literature. Wilson (1919) distinguished between "young" and "adult" copepod parasites by shape, size, and colour, and mentioned that ova in ovaries of individual *Sphyrion* were always similar in size. Mr R. C. Knapp-Fisher in unpublished work at this Station defined stages of maturity of *S. lumpi* by external examination for size, shape, and condition. In defining "immature," "maturing A and B," "mature," and "spent" he assumed that eggsacs grew over a more or less extended period as trunk contents (ova) grew and were extruded completely into them, and that a flattened "empty" trunk was in a "spent" condition. He intimated in this way that the parasite on maturation of its ova spawned once and that after the embryos hatched it died. Templeman and Squires (1960) also intimate this in the statement "After the larvae which develop in the ovisacs are hatched out and the ovisacs are eventually lost the trunk shrivels, its contents degenerate and the animal dies." Heegaard (1947), however, found that on keeping a female parasitic copepod, *Lernaeocera branchialis*, alive for 2 months in an aquarium, it produced eggsacs full of eggs on two occasions. The embryos on each occasion all matured simultaneously and hatched when the eggsacs split open and were lost. Templeman and Fleming (1963) suggested that *L. branchialis* may spawn in nature several times during the relatively long life of the adult.

[1]Received for publication August 30, 1965.

J. Fish. Res. Bd. Canada, 23(4), 1966.
Printed in Canada.

MATERIALS AND METHODS

The 263 specimens of *S. lumpi* examined were taken from *Sebastes marinus* s.l. captured in exploratory fishing during May–November 1947–53, by the *Investigator II* in Newfoundland and Labrador waters. Two other workers had measured the specimens previously. Although all were examined to find out if males were present, only 159 were used for remeasuring the diameters of ova since the rest had been embedded for histological preparations.

The lengths and widths of the trunks and lengths and diameters of the eggsacs (Fig. 1) were measured with vernier calipers. Since some large wide specimens may not be quite as long as small long ones the combined values of width and length (diameters of a subcircular surface) were used to give a better indication of size than length or width alone. "Size" referred to in this paper means length plus width of the trunk of a specimen.

The trunks of all specimens were cut open and the diameters of a few ova, if any were found, were measured on a Turtox millimetre grid or with an ocular micrometer under magnification of about 60×. Eggs from eggsacs, if present, were measured in the same way. After histological preparation, ova were found to have shrunk by 0.04 to 0.10 mm and eggs in eggsacs by 0.07 to 0.20 mm. Egg counts were made from a number of 3 mm lengths of eggsac cut evenly from different parts of the eggsac, and the total number of eggs calculated from average number of eggs per millimetre of eggsac. Only eggsacs with eggs about 0.4 mm in diameter were used to estimate fecundity and only 12 females with this size of egg and complete eggsacs were available for counting.

A number of females of representative sizes and stages of maturity, and immature and adult males, were dehydrated and embedded in hard wax, sectioned at 10 μ, and stained with Mallory's trichrome. Some males were cleared in cedar oil and mounted whole in balsam. One was stained in chlorazol black and some in azocarmine. Whole mounts of some females were made in damar gum after dehydration and clearing in xylol.

FEMALES

Maturity

In ovigerous females, as the embryos in the eggsacs developed and came close to hatching, the ova in the ovaries increased in diameter (Fig. 2). When eggs had apparently just been extruded into the eggsacs (yolk content was high and little differentiation of the blastodisc was apparent), the diameter of ova in the ovaries was 0.1 or 0.2 mm (Fig. 2, I, A and B), but when the embryos were ready for hatching the ova diameters were 0.4 mm (Fig. 2, III, A and B). Egg diameters in the eggsacs shortly after extrusion were 0.4 mm. This parallel development of embryos in the eggsacs and ova in the ovaries indicates that when the embryos had hatched and the old eggsacs were lost, a new batch of eggs would be ready for extrusion into new eggsacs.

It was possible to define arbitrary stages of maturity from diameters of ova in the ovaries and the stage of development of embryos when eggsacs

were carried. No clear exception to this simultaneous development of ova and embryos was found. The stages of maturity (Table I) may be defined as follows:

STAGE I. JUVENILE. Ova were proliferating as oocytes (Fig. 3A). Size did not exceed 20 mm.

STAGE II. IMMATURE. Ova were too small to be seen with the dissecting microscope. These and the following stages occurred in specimens varying in size from 21 to 45 mm.

STAGE III. LATE MATURING, NONOVIGEROUS. Ova were very small, 0.1 or 0.2 mm in diameter (Fig. 3B).

STAGE IV. EARLY MATURING, NONOVIGEROUS. Ova were 0.3 or 0.4 mm in diameter, the larger in some instances ready for extrusion (Fig. 3C).

STAGE V. LATE MATURING, OVIGEROUS (FIG. 2, I AND II). The ova in the ovaries were 0.1 or 0.2 mm in diameter and the eggs in eggsacs showed an early stage of embryonic development.

STAGE VI. MATURE OR EARLY MATURING, OVIGEROUS (FIG. 2, III). The ova in ovaries were 0.3 or 0.4 mm, the eggs in eggsacs were 0.5 mm in diameter, and the embryos were ready to hatch.

CEMENT GLANDS, OVIDUCTS, AND BODY MUSCLES

The gross anatomy of the cement glands, oviducts, and body muscles in *S. lumpi* (Fig. 4–6) indicates that a copious supply of cement would be available for formation of eggsacs, eggs would have free egress through the oviducts, and the many bundles of striated body muscles would act quickly to accomplish spawning over a very short period. The cement gland (CG) is a cylindrical style or rod rounded at the anterior and flattened at the posterior end. In appearance it is composed of numerous transverse sections closely compacted like the leaves of a closed book. It is attached along the thin inner wall of the oviduct, which completely surrounds it.

The oviducts (OV) are thick-walled along the outer edge of the curve they form close to the trunk wall. The tissues of the thick-walled portion are glandular rather than muscular although there are apparently some smooth muscle inclusions. Each oviduct has one small oval-shaped opening near the anterior end through which the eggs will pass into a wide canal. This leads to the external opening of the oviduct (Fig. 6,O). The eggsacs are formed at the external opening as the eggs are extruded.

Numerous striated muscle bundles (Fig. 6,M) are attached to the opposing dorsal and ventral walls. Occasional specimens had contracted muscles and were thin and flat in appearance, while others had relaxed muscles and were subspherical in shape. In the relaxed condition the volume of the trunk cavity would be comparatively large.

TABLE I. Stages of maturity in *S. lumpi* as indicated by ova diameters at different trunk sizes of females[a] with or without eggsacs. Stages are indicated as defined in text.

Trunk size (length + width) (*mm*)	Number without eggsacs				Number with eggsacs		Number of males found on females	
		Diameter of ova in ovary, *mm*			Diameter of ova in ovary, *mm*			
	Oocytes only	Very small or none seen	0.1, 0.2	0.3, 0.4	(Early embryos in eggsacs) 0.1, 0.2	(Late embryos in eggsacs) 0.3, 0.4	Immature	Adult
Maturity stage:	I	II	III	IV	V	VI		
6–10	5	...	...	...	...	...	...	...
11–15	3	...	...	...	...	...	...	...
16–20	12	...	...	...	...	...	...	2
21–25	...	5	9[b]	7[c]	1	...	2	0
26–30	...	1	14	22	5[d]	1	1	4
31–35	...	4	3	17	8	10	1	3
36–40	...	2	3	4	5	6	...	...
41–65	...	1	1	4	1	4	...	...
46–50	...	...	...	...	1[e]	...	...	...
Total:	20	13	30	54	21	21	13	
Per cent:	13	8	19	34	14	13	5[f]	

[a]Based on 159 females which could be remeasured.
[b]One with length + width = 19 mm.
[c]One with length + width = 20 mm.
[d]Two with ova diam. 0.3 mm.
[e]One with ova diam. 0.3 mm.
[f]Per cent occurrence on the 263 females examined.

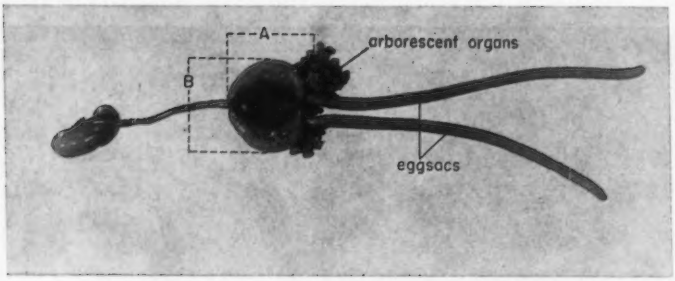

FIG. 1. Ovigerous female *Sphyrion lumpi* showing trunk measurements (A = length, B = width) and general appearance when dissected out of a redfish.

FIG. 2. Transverse sections of ova in trunks (A) and eggs in eggsacs (B) of ovigerous females. In each of I, II, and III the trunk and eggsac belong to the same female. In I the ova are small while the eggs in eggsac show little differentiation. In II the ova are medium in size and the eggs show some differentiation of the blastodisc. In III the ova are large while the eggs show well developed embryos almost ready to hatch.

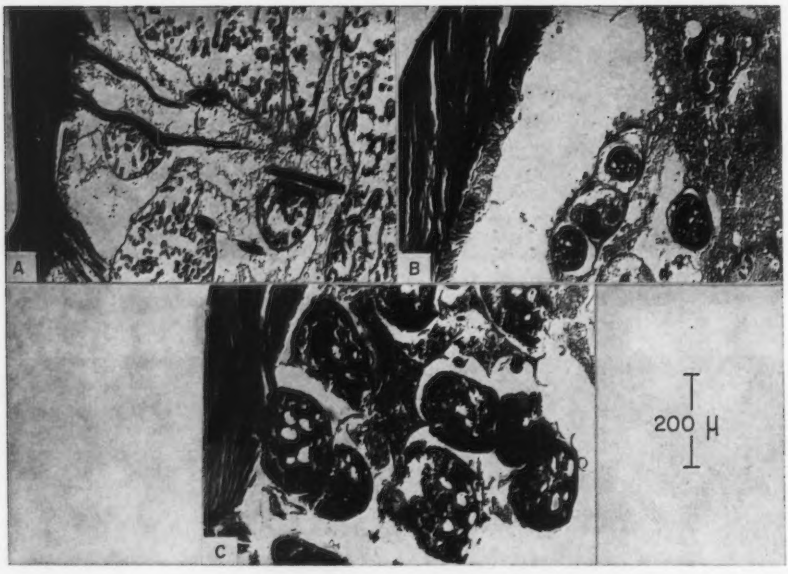

FIG. 3. Transverse sections of trunks of nonovigerous *Sphyrion lumpi* females. A = juvenile, with ova proliferating as oocytes; B = late maturing with ova 0.1 mm in diameter, and C = early maturing with ova 0.3 or 0.4 mm in diameter (Some shrinkage of ova has taken place; see text).

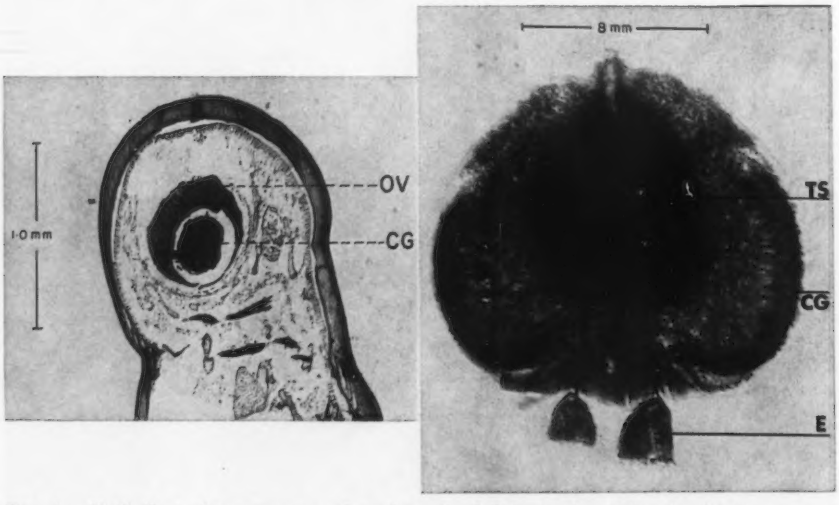

FIG. 4. (*Left*) Part of transverse section of trunk of *Sphyrion lumpi* female showing cement gland (CG) and oviduct (OV).

FIG. 5. (*Right*) Unstained whole mount of *Sphyrion lumpi* female cleared in xylol and mounted in balsam. TS = approximate position of transverse sections as in Fig. 2, 3, and 4; CG = cement gland; and E = part of eggsac.

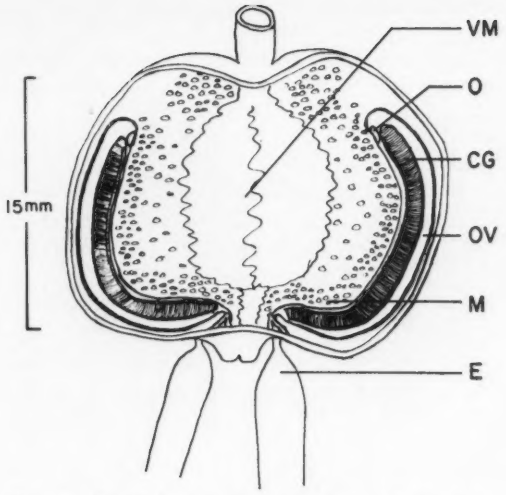

FIG. 6. Semidiagrammatic representation of gross internal anatomy of trunk of female *Sphyrion lumpi*. VM = visceral mass, O = opening of oviduct, CG = cement gland, OV = oviduct, M = muscle bundles, E = eggsac.

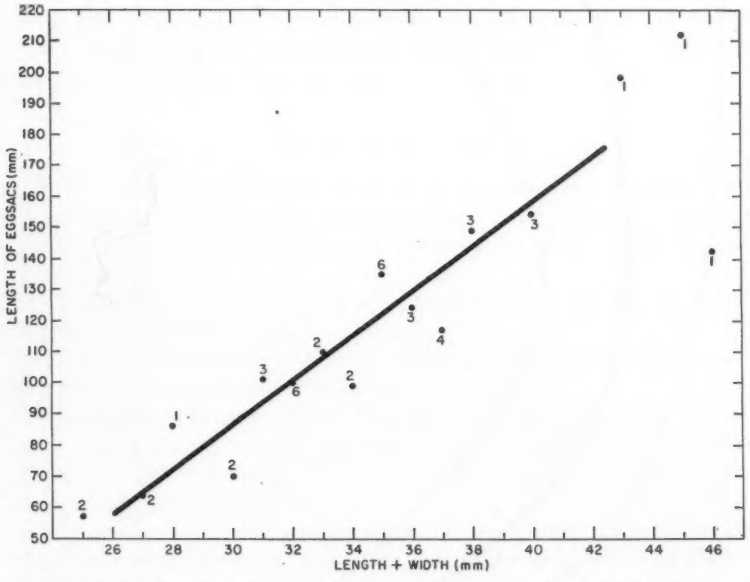

FIG. 7. Freehand curve of total length of both eggsacs carried by 42 *Sphyrion lumpi* females at different trunk sizes (length + width). Number of individuals measured at each point is shown.

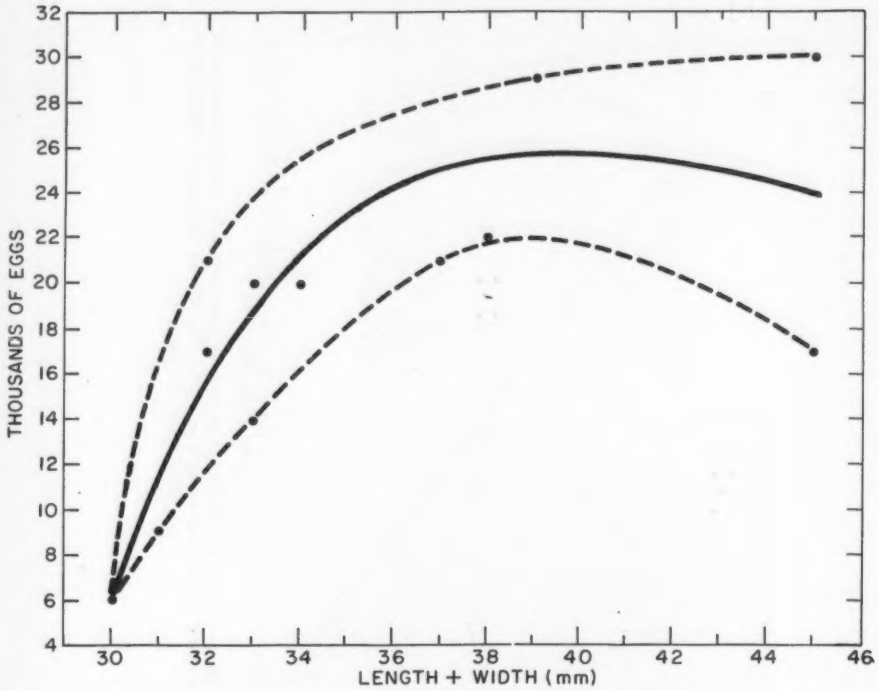

FIG. 8. Freehand curves of limits and central tendency of calculated numbers of eggs at each trunk size (length + width) of *Sphyrion lumpi* females. Though counts were made from eggsacs of 23 females, calculations were based on counts from 12 females which had both eggsacs intact.

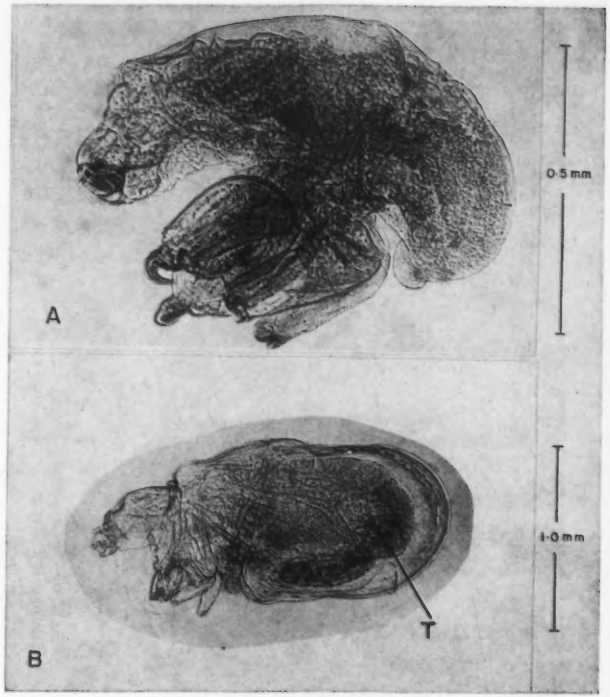

FIG. 9. Whole mounts of *Sphyrion lumpi* males stained in azocarmine and cleared in xylol. A = immature male 0.8 mm in total length; B = mature male 2.0 mm in total length; T = part of spermatophore receptacle.

In Mallory's trichrome the cement glands and striated body muscles stained red, and the oviducts purple.

Fecundity

Eggsacs varied in length directly with the size of the trunk from about 60 mm (combined length of two eggsacs) at a trunk size of 26 mm to about 200 mm at a trunk size of 45 mm (Fig. 7). The increase in eggsac length appeared to be a function of the increase in trunk volume and this was concomitant with an increase in the number of eggs. Estimates of egg numbers ranged from 6000 eggs at a trunk size of 30 mm to 30,000 eggs at a trunk size of 45 mm (Fig. 8). Eggs were moderately compacted in the eggsacs, which varied in diameter from 2.3 to 3.2 mm. Average number of eggs per millimetre length of eggsac varied from 90 to 200 eggs.

MALES

Nine mature and four juvenile males were found on 12 of the 263 females examined (Table I). Some of these were still attached by the large hooks of the maxillipeds to the arborescent organs, where they were presumably feeding parasitically since the mouth parts of some were inserted into and possibly extracting fluid from the arborescent organs. The mature males (1.8–2.1 mm in length) had large abdomens and fully developed testes and seminal receptacles (Fig. 9 B) while the juveniles (0.8 mm in length) had only small abdomens and the reproductive organs could not be readily seen (Fig. 9 A).

GENERAL DISCUSSION

Multiple spawning is important to the survival of this parasite, which is faced with considerable hazard in propagation. Many eggs or many batches of eggs need to be produced to ensure maintenance of its numbers. Some of the hazards are:

(a) The difficulty of a weak-swimming metanauplius to find and become attached to a mobile target — a redfish.

(b) The difficulty, presumably greater than (a), for a male metanauplius to find and become attached to a female *S. lumpi*.

(c) The probable shortness of the free-swimming period in the life of this copepod (we have no exact knowledge of the length of this period in *S. lumpi* although it has been found to be short (36 hr) in some other Lernaepodidae (Wilson, 1911)).

(d) The possibility of loss of one or more batches of eggs because no male is available to fertilize them. Relatively few males were found on the sample examined, although some may have been lost through at least two examinations during which males were not looked for. However, they had been lost in nature from most of the ovigerous females and would, therefore, most probably copulate with a female only once. Sperm storage in the

female was not demonstrated by the techniques used. If sperm storage does occur more females should have been ovigerous. Only 27% of those examined were ovigerous but 80% were potentially ovigerous (Table I), although about half of these would probably be ovigerous for the first time.

(e) The number of eggs produced in a single batch is comparatively low (6–30 thousand in the specimens examined (Fig. 8)).

At 6–8 C the observed incubation period of eggs of *Lernaeocera branchialis* was 15 days (Heegaard, 1947). At temperatures of 3–6 C on redfish grounds, the incubation period of *S. lumpi* eggs would most probably be longer than this, although Heegaard suggests that the incubation period in most parasitic copepods would not exceed 3–4 weeks. Also, since the adult reproductive phase of *Lernaeocera* is about 1 year or longer (Kabata, 1958; Scott, 1929), it is possible that *S. lumpi* may live for a corresponding period. Kelly and Barker (1964) suggest that in the Gulf of Maine *S. lumpi* may live for one or more years.

ACKNOWLEDGMENTS

The lengths and widths of trunks and lengths of eggsacs were measured by Mr R. C. Knapp-Fisher and corrected by Miss Elizabeth Scott (both formerly employed at this Station). Most of the histological preparations were made by Mr G. E. Tucker. The photographs were made by Mr E. L. Rowe. Messrs Arthur May, E. J. Sandeman, and V. M. Hodder made helpful suggestions for improvement of the manuscript.

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Glycogenolytic Enzymes of Cod (*Gadus callarias*) Muscle[1]

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ABSTRACT

Some properties of the enzymes amylase, phosphorylase, and phosphoglucomutase in homogenates of cod muscle have been investigated. Amylase is activated by a short period of autolysis and by chloride ions; hydrolyses cod muscle glycogen at about half the rate it hydrolyses mussel glycogen; has an optimum temperature of 52 C for up to 5 hr incubation; and has a wide range of activity with maxima around *p*H 5.1 and between *p*H 7.0 and 7.5. Using phloridzin, an estimate of the relative importance of hydrolytic and phosphorolytic cleavage of polysaccharide in cod muscle homogenates has been made. Phosphorylase shows two activity maxima at *p*H 5.5 and 6.7. The properties of phosphoglucomutase, with the exception of its *p*H activity, in cod muscle are similar to those reported for this enzyme in other tissues. Cod muscle phosphoglucomutase has maximum activity between *p*H 8 and 9.

INTRODUCTION

The experiments described below were carried out during an attempt to ascertain the proportions of glycogen degraded postmortem in cod muscle by phosphorolysis and by hydrolysis. The former method of degradation would give rise to sugar phosphates and eventually to pyruvic and lactic acids, while the latter would produce lower saccharides and eventually maltose, which in turn may be cleaved to give glucose. It is now well established that both these pathways operate in the muscle of fish of various species (see Burt (1962) for review to 1961; Tarr and Leroux, 1962; Jones, 1962a; Andreyev, 1962; Nagayama, 1961 a,b,c,d; MacLeod et al., 1963; McGeachin and Debnam, 1960; Martin and Tarr, 1961) but in this study attention has been focussed on those enzymes (phosphorylase and amylase) which catalyse the initial steps of each pathway.

Japanese sea bass (*Lateorabrax japonicus*) muscle contains a glycogen phosphorylase which is somewhat unstable during postmortem storage and it is pointed out that fresh fish have to be used as sources of this enzyme (Ono et al., 1957). A later report from the same laboratory (Nagayama, 1961b) indicates that this loss of activity on storage is not as complete as was thought formerly. Andreev (1958) on the other hand reported an autolytic liberation of amylase in fish muscle extracts at room temperature. This possible difference in the properties of these two enzymes was thought to introduce some

[1]Received for publication September 13, 1965.

J. Fish. Res. Bd. Canada, 23(4), 1966.
Printed in Canada.

difficulties in the way of determining the activities of both enzymes in the same muscle preparation, but by the use of an inhibitor of phosphorylase an approximate estimate of the relative amounts of amylase and phosphorylase activities has been made.

In addition, a fairly extensive study of the properties of cod muscle amylase is reported here, as are the results of some experiments on the properties of phosphorylase and phosphoglucomutase.

METHODS

Polysaccharide determinations. The concentration of starch in deproteinized incubates (reaction mixtures) was determined by measuring optical density at 600 mμ after the addition of iodine (0.055 N) in potassium iodide solution (2.5%). Glycogen was determined by the procedure of Krisman (1962).

Reducing sugar determinations. Somogyi's (1945) procedure was used.

Phosphate determinations. The procedure of King (1932) was used to determine inorganic phosphate and, after heating for 7 min in N sulphuric acid at 100 C, acid-labile phosphate concentrations.

Removal of protein from incubates. Barium hydroxide–zinc sulphate treatment (Somogyi, 1945) was used when reducing sugar levels were subsequently to be determined, and treatment with trichloroacetic acid (10%) was used prior to determination of polysaccharides.

Inhibitors and activators. The effects of phloridzin on starch breakdown; of chloride ions, ammonium molybdate, and dialysis on amylase activity; and of magnesium ions, cysteine, cupric ions, zinc ions, and fluoride ions on phosphoglucomutase activity were investigated.

Incubates. The composition of the incubates used for each of the following experiments is detailed below. Muscle excised from a freshly killed, aquarium-maintained cod was used for several of the experiments if they were to be carried out the same day. The unused parts of the fish muscle were cut into portions, wrapped in aluminium foil, and kept frozen at –30 C for use on later days. Toluene was added to homogenates as a bacterial inhibitor. Frequent checks by microscopic examination were made to ensure that bacteria were absent from incubates.

Care was taken during the homogenizing of the muscle to avoid excessive foaming and heating up of the sample. Grinding the muscle to a paste with its extractant in a mortar (Andreev, 1958) was used in the preparation of the amylase autolysates. An electric homogenizer was used for the other enzyme preparations.

RESULTS AND DISCUSSION

Amylase Activity

AUTOLYTIC LIBERATION

From previous reports on the properties of amylase in fish muscle (Andreev, 1958) it was expected that a period of autolysis would be necessary before amylase activity could be detected in extracts or homogenates of fish

muscle. The results of a preliminary survey of various conditions of autolysis are summarized in Table I and these confirm the necessity for such autolysis. Cod muscle (20 g) was homogenized with water (40 ml) and toluene (1 ml), and aliquot portions were subjected to the various types of treatment listed in the Table. A series of tubes was set up containing β-glycerophosphate buffer (0.5 M; *p*H 7.1; 10 ml), glycogen solution (1%, 3 ml) sodium chloride (100 mg), and homogenate (5 ml) for incubation at 35 C. Aliquots (2 ml) were removed at zero time and after 3 hr for determination of reducing sugar. The results are expressed in terms of the reducing sugar increase during 3 hr relative to that produced (730 μg apparent maltose/2 ml incubate) by the most active preparation. It was also confirmed that centrifugation of the homogenate at approximately 2000 g for 10 min does not remove any amylase activity with the residue whether such centrifuging is done before or after the autolysis.

TABLE I. Amylase activity as affected by various conditions of autolysis.

Time and temperature of autolysis	Relative activity
3 hr at 37 C followed by 18 hr at 0 C	100
3 hr at 18 C followed by 18 hr at 0 C	53
21 hr at 18 C	38
21 hr at 0 C	20
2 hr ultrasonic treatment[a] at 0 C followed by 18 hr at 0 C	15
Homogenized fresh	11

[a]An M.S.E. ultrasonic disintegrator operating at 18,000–20,000 cps and 60 w output was used.

These results have given rise to the speculation that cod muscle amylase may be bound to protein (Andreev, 1958) although the association of the enzyme with some subcellular particles, such as lysosomes, would also fit this pattern.

ACTIVATION AND INHIBITION

The activation of this amylase by chloride ions was demonstrated. Frozen cod muscle (80 g) homogenized with water (160 ml) and toluene (5 ml) was allowed to autolyse for 3 hr at 18 C. Aliquot portions were taken and dialysed against water at 5 C for varying lengths of time. The remainder was kept at the same temperature as a control, and portions were removed from it for assay of amylase activity in parallel with the dialysed homogenate. The assay mixture used was of the following composition: β-glycerophosphate buffer (0.1 M; *p*H 7.1; 10 ml), starch (1%, 2 ml), and homogenate (5 ml). Duplicate tubes were set up in the case of the dialysed homogenate. To half of these and to the controls, sodium chloride (100 mg) was added. Aliquots

(1 ml) were removed for determination of starch and of reducing sugar concentrations. Table II shows the amount of starch hydrolysed by these homogenates relative to that hydrolysed during 2 hr incubation by the undialysed control after 17 hr storage. Very similar results were obtained when the corresponding increases in reducing sugar concentrations were determined. As a check on the efficiency of the dialysis, the concentration of inorganic phosphate in the dialysing homogenate was determined and the relative values found are also given in Table II.

TABLE II. Effect of dialysis and sodium chloride on amylase activity.

	Relative hydrolysis of starch				Relative increase in reducing sugar			
Time of dialysis (*hr*)	17	41	65	89	17	41	65	89
	1 hr incubation				*2 hr incubation*			
Undialysed controls + NaCl	61	69	66	65	58	81	69	54
Dialysed extract + NaCl	58	64	67	59	62	69	77	62
Dialysed extract	47	38	45	37	12	8	12	12
	2 hr incubation				*4 hr incubation*			
Undialysed controls + NaCl	100[a]	124	110	103	100[b]	135	108	92
Dialysed extract + NaCl	98	111	119	92	100	110	–	92
Dialysed extract	67	74	67	57	33	27	35	27
Inorganic phosphate concentration as percentage of concentration in control	5.6	0.34	0.24					

[a]Equivalent to a reduction of 77% of the initial starch-iodine colour.
[b]Equivalent to an increase of 360 μg maltose/ml incubate.

Ammonium molybdate (0.3%) was found not to inhibit cod muscle amylases. Two incubation mixtures similar to the undialysed controls for the chloride activation experiment were set up. To one ammonium molybdate was added to give a final concentration of 0.3%. Aliquots (1 ml) were removed at intervals for determination of starch. After 1 hr, 78 and 69% of the initial starch-iodine colour had been lost from the incubates, respectively, without and with added molybdate. After 2 hr, both mixtures had lost 82% of the initial starch-iodine colour.

ACTIVITY TOWARD DIFFERENT SUBSTRATES

The action of this crude amylase preparation on two different glycogens was measured. Glycogen isolated by alcohol precipitation from a hot water

extract of mussel (*Mytilus edulis*) was hydrolysed at 2.2 times the rate at which glycogen prepared by alcohol precipitation from a cold trichloroacetic acid extract of cod muscle was hydrolysed. An homogenate of muscle (20 g) in water (40 ml), and toluene (1 ml) was allowed to autolyse for 2 hr at room temperature. Incubation tubes containing centrifuged homogenate (5 ml), water (10 ml), glycogen solution (3 ml), and sodium chloride (100 mg) were set up and aliquots (2 ml) removed for reducing sugar determination after 0 and 3 hr at 36 C. The increases in apparent maltose concentration were 90 and 200 μg/2 ml incubation mixture for the tubes containing cod muscle and *Mytilus* glycogen, respectively.

In an attempt to find the reason for the relatively slow rate of amylolysis of the cod muscle glycogen, a sedimentation velocity run was made in a Spinco Model E ultracentrifuge. This glycogen in 0.5% aqueous and salt (0.1 M NaCl) solution was found to be monodisperse with an $S_{20,w} = 142 \times 10^{-13}$ and 145×10^{-13}, respectively. Assuming a partial specific volume of 0.62 (Greenwood, 1952) and a D_{20} of 1.5×10^{-7} cm^2 sec^{-1} (Bell et al., 1948) an approximate molecular weight of 6.2×10^6 for cod muscle glycogen has been calculated. This is considerably higher than the value of 3.8×10^6 reported for the glycogen of *Mytilus edulis* (Manners, 1957) and could therefore account, in part at least, for the different rates of breakdown. On the other hand, *Mytilus* glycogen and starch were hydrolysed at approximately the same rate.

EFFECT OF TEMPERATURE AND *p*H

Figure 1 illustrates the effect of temperature on the activity of cod muscle amylase. Enzyme preparations similar to those described above were used. The results of two experiments are given; one using cod muscle glycogen (5 hr incubation) as substrate and the other *Mytilus* glycogen (2½ hr incubation). Incubation was carried out at *p*H 7. The rather high optimum temperature found was not entirely unexpected in view of the fact that occasionally a certain amount of amylase activity remained in extracts that had been heated on a boiling water bath for use as controls. More prolonged heating destroyed the activity completely, however. Andreev (1958) reported a 37–42 C temperature optimum for 2- and 3-hr incubations with an aqueous extract of fish muscle.

The amylolytic activity of aqueous extracts of cod muscle was found to be maximal at *p*H 7.0–7.5, but to be fairly active over the range of *p*H 6.0–8.5. This is similar to the *p*H-activity properties of the amylase of carp and shad muscle (Andreev, 1958).

ATTEMPTED QUANTITATION OF AMYLASE:PHOSPHORYLASE RATIOS

An attempt to measure the relative amounts, and how they might be affected by *p*H, of hydrolysis and phosphorolysis catalysed by an extract of cod muscle is illustrated in Fig. 2. Nonautolysed extracts were used to simulate more closely the conditions present in whole cod muscle and also to obviate the possible inactivation of phosphorylase. Phloridzin is a known inhibitor of phosphorylase activity (Cori et al., 1955) but two drawbacks to its use in

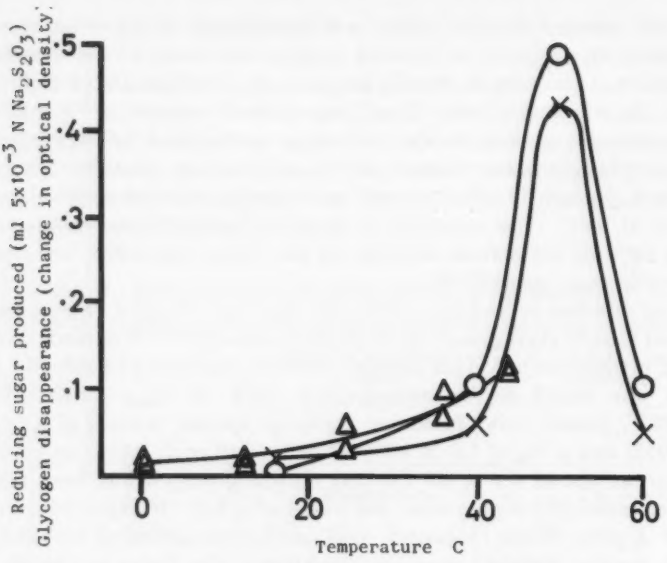

FIG. 1. *p*H-activity curve for amylase of cod muscle. △ Substrate *Mytilus* glycogen. 2½ hr incubation. Reducing sugar increase assayed; ○ Substrate cod muscle glycogen. 5 hr incubation. Glycogen decrease assayed; × Substrate cod muscle glycogen. 5 hr incubation. Reducing sugar increase assayed.

these experiments were discovered. Both phloridzin and its aglucone phloretin interfered with the colorimetric determination of starch. This interference was counteracted by the use of higher iodine concentrations. Also, the cod muscle extracts used in these experiments appeared to contain high activities of a glucosidase which released glucose from phloridzin. The large increases in reducing value, obtained as a result of this reaction, overlying those produced by the enzymatic hydrolysis of polysaccharide have made the interpretation of some results rather difficult. Cod muscle (140 g) was homogenized with potassium chloride solution (0.4%; 400 ml) and the debris removed in a centrifuge. For incubation at *p*H values between 4.0 and 5.1 an acetate buffer (0.2 M) containing phosphate (0.2 M) was used and for *p*H values between 5.5 and 8.0 a phosphate (0.2 M) buffer was used. Tubes (four for each *p*H value) containing buffer (10 ml) and starch solution (0.5%; 2 ml) were set up in a water bath at 37 C. To half of the tubes phloridzin (60 mg) was added. Reaction was started by the addition of extract (8 ml) and tubes were removed for determination of starch concentration at zero time and after 2 hr incubation.

Comparison of these two curves with each other and with that obtained from a similar experiment in which the increase in reducing power was followed in the absence of phloridzin shows that in cod muscle there are two optimum *p*H values (around 5.5 and 6.7) for phosphorylase activity. Measurement of increases in reducing power was limited to the *p*H region of practical interest.

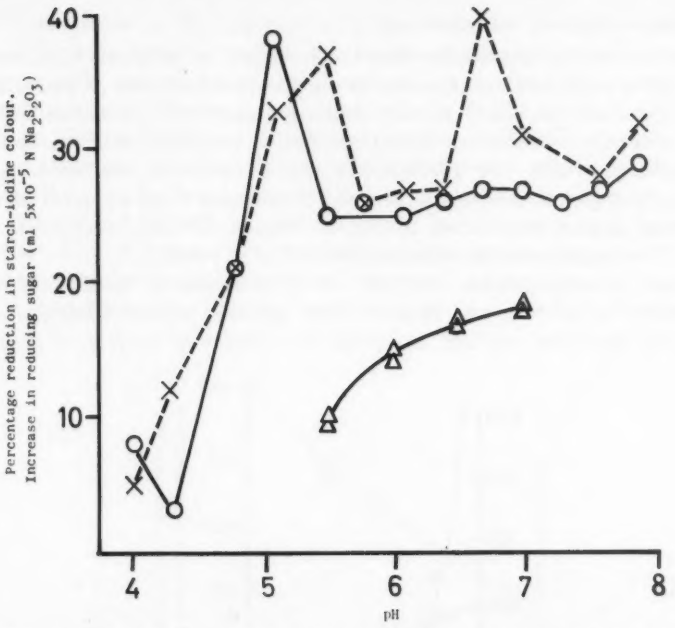

FIG. 2. Effect of *p*H on relative activities of amylase and phosphorylase in an extract of cod muscle. △ Increase in reducing sugar. No phloridzin; × Loss of starch. No phloridzin; ○ Loss of starch. Phloridzin present.

In addition to the broad peak (maximum *p*H 7.0) for hydrolase activity there is another component with a fairly sharp maximum at *p*H 5.1. This latter and the phosphorylase active at the lower *p*H are unlikely to be of importance either during the life of the fish or during postmortem storage (when *p*H 5.5 is only exceptionally reached). There is a slight possibility that the picture may be confused by the fact that the buffer used from *p*H 5.1 down was different from the one used from *p*H 5.5 up. Since the concentration of inorganic phosphate was constant throughout the *p*H range, the conclusion that a phosphorylase active at *p*H 5.5 is present is likely to be correct. However, without further evidence, the same may not be said for the amylase active at *p*H 5.1.

Consideration of the relative amounts of glucose and glucose 6-phosphate which appear during postmortem storage of cod muscle (Burt, 1961) drew attention to the possible importance of hydrolysis in the degradation of glycogen. It would be unwise to extrapolate the results of the experiments on muscle extracts and homogenates reported here in order to predict the state of affairs in whole muscle. However, the results given above have at least proved the presence in cod muscle of enzymes capable of hydrolysing a large proportion of the glycogen present at death. The relative importance of hydrolysis in total glycogenolysis is likely to vary markedly with *p*H and from the data in Fig. 2 is shown to fall from 87% at *p*H 7.0 to 67% at *p*H 6.7.

ACTION PATTERN OF AMYLASE

To rule out the possibility that the increases in reducing value measured in the above experiments were due to the combined actions of phosphorylase, phosphoglucomutase, and glucose 6-phosphatase, the corresponding values for the intensity of colour of the starch-iodine complex (as a per cent of the value obtained with the undegraded starch) and for the reducing value (in terms of apparent per cent hydrolysis to maltose) from a typical incubation are plotted against each other in Fig. 3. The curve thus obtained is characteristic of an α-amylase degradation (Bourne et al., 1945).

Paper chromatographic analysis of the products of incubation revealed the presence of glucose and maltose, with glucose predominating. Andreyev (1962) has described maltase activities in extracts of muscle of several fish

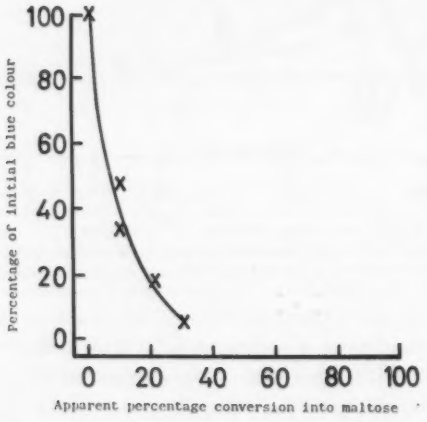

FIG. 3. The hydrolysis of starch by cod muscle amylase.

species, while Tarr and Leroux (1962) have reported the appearance of labelled glucose and maltose after incubation of radioactive glycogen with an homogenate of salmon muscle. Maltose has not been detected in extracts prepared from muscle of chill-stored cod (Burt, 1961) so it might be supposed that this fish also has high maltase activity in its muscle. It is not known, however, if this is the same enzyme that is responsible for the hydrolysis of phloridzin that was observed in some of these experiments.

PHOSPHORYLASE ACTIVITY

Initial attempts to demonstrate, by following glycogen breakdown and a decrease in inorganic phosphate, the presence of phosphorylase in cod muscle homogenates revealed very low levels or none at all. However, when the kinetically more favourable reverse reaction (i.e. synthesis of glycogen from

glucose 1-phosphate in the presence of polysaccharide "primer") was tested for, phosphorylase activity was readily apparent. Adenosine monophosphate was intentionally not added to the homogenates so that the status within the intact muscle might be better simulated. It is known that cod muscle contains an active adenosine 5′-monophosphate deaminase (Jones, 1962b) and it can therefore be assumed that concentrations of adenosine monophosphate are likely to be low in these enzyme preparations. Consequently the enzyme phosphorylase will be predominately in the inactive *b* form (Cori et al., 1955). Figure 4 shows the change in concentration with time of glucose 1-phosphate and inorganic phosphate (expressed as per cent of the initial glucose 1-phosphate value) during incubation at 36 C of mixtures containing

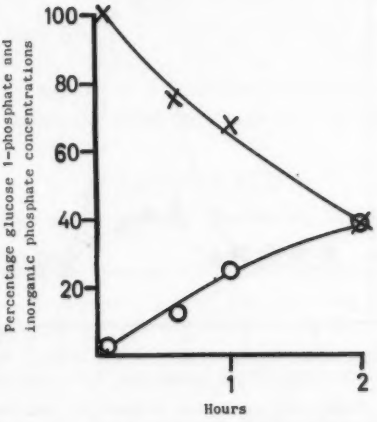

FIG. 4. Changes in glucose 1-phosphate (×) and inorganic phosphate (○) concentrations catalyzed by cod muscle extract expressed as percentage of initial glucose 1-phosphate concentration (phosphorylase activity).

glycogen primer (0.4%; 1 ml) and glucose 1-phosphate (2×10^{-3} M; 1 ml) with buffered cod muscle extract containing cysteine (2 ml). The extract was prepared by diluting the supernatant (3 ml) from a centrifuged homogenate of cod muscle (25% w/v) in 0.4% potassium chloride solution with β-glycerophosphate buffer (0.07 M; *p*H 6; 50 ml) containing cysteine (0.06 M). The increase in inorganic phosphate is equivalent to over half the amount of glucose 1-phosphate lost; i.e. is the result of phosphorylase action, as controls without glycogen primer showed little phosphatase activity under these conditions. The rest of the glucose 1-phosphate could have been lost as a result of phosphoglucomutase action, despite the comparatively low *p*H and the absence of added magnesium ions (see below) as quite a protracted incubation period was used.

PHOSPHOGLUCOMUTASE

The probable presence of phosphoglucomutase activities in the preparation used to demonstrate phosphorylase activity rendered an investigation of this enzyme's properties relevant to this study. The properties of phosphoglucomutase in seabass (Nagayama, 1961c) and in lingcod (Martin and Tarr, 1961) muscle have been investigated and the properties (with the exception of its *p*H-activity) of the cod muscle enzyme were shown to be very similar.

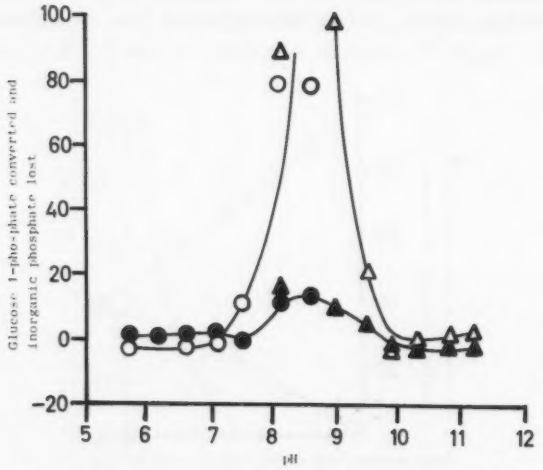

FIG. 5. *p*H-activity curve for phosphoglucomutase of cod muscle. Glucose 1-phosphate converted in Tris-maleate (○) and glycine–NaOH (△) buffer. Inorganic phosphate lost in Tris-maleate (●) and glycine–NaOH (▲) buffer. Values expressed as percentage of initial glucose 1-phosphate concentration.

Cod muscle phosphoglucomutase is readily extracted into aqueous and dilute salt solution, is activated by magnesium ions, shows enhanced activity in the presence of cysteine, and is inhibited to the extent of 95, 98, and 35%, respectively, by cupric, zinc, and fluoride ions (3×10^{-3} M) during incubation for 15 min at *p*H 8 and 35 C.

Preliminary experiments had shown that the optimal *p*H for phosphoglucomutase activity of these cod muscle extracts lay somewhat higher than the 7.5 that had been expected from consideration of the value quoted by Martin and Tarr (1961) for lingcod muscle phosphoglucomutase. Accordingly, a series of tubes of the following composition was set up for incubation at 37 C: tris-maleate or glycine-sodium hydroxide buffer (0.2 M; 3 ml) (Gomori, 1955), magnesium chloride (10^{-3} M; 1 ml) and extract (1 ml of the supernatant from a centrifuged homogenate of 7 g cod muscle in 200 ml 0.4% potassium chloride solution). Glucose 1-phosphate (2×10^{-2} M; 1 ml) was added to start

the reaction and 2 ml of 10% trichloroacetic acid solution added after 10 min to stop it. The tubes were centrifuged, the residue washed with a further 2 ml of 10% trichloroacetic acid, and re-centrifuged. The supernatants were combined and aliquots taken for determination of orthophosphate and of acid-labile phosphate. Zero-time controls were obtained by adding the trichloroacetic solution before the glucose 1-phosphate. A parallel series of tubes was set up to determine the actual *p*H of each incubation mixture at 37 C. This also made allowance for the alteration of the *p*H of the buffer by the extract. Figure 5 shows that phosphoglucomutase has maximum activity between *p*H 8 and *p*H 9. There is no apparent reason for the loss of inorganic phosphate during incubation at *p*H values between 7 and 10.

ACKNOWLEDGMENTS

Dr J. J. Connell and Mr P. F. Howgate carried out the glycogen sedimentation runs. Miss M. N. I. Barclay, Department of Biochemistry, University of Glasgow, during the tenure of a vacation studentship at Torry Research Station, determined the temperature optimum for cod muscle amylase.

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The Advent of the Spring Bloom in the Eastern Subarctic Pacific Ocean[1]

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ABSTRACT

The spring phytoplankton bloom in the eastern subarctic Pacific Ocean was described from estimations of the critical depth and the depth of the mixed layer. The results suggested that the spring bloom begins during February in the area south of 45°N and east of 135°W. During March the bloom area advances in a northwesterly direction to 50°N at 125°W and 45°N at 135°W. A net increase in primary production is also possible during March near 55°N and 155°W. During April, the spring bloom is generally well established throughout the region except in a central area where suitable conditions are not firmly established until May. This description is supported by the distribution of copepods in the region during April.

INTRODUCTION

Conditions for the onset of the spring phytoplankton bloom in temperate latitudes have been shown to be largely dependent both on the stability of the water mass and on the penetration of sufficient light to cause a net increase in production. One approach to an examination of these conditions was suggested by Gran and Braarud (1935) and developed into a prediction model by Sverdrup (1953). Studies using the model have been carried out by Marshall (1958), Semina (1960), and Cushing (1962). A similar type of study was carried out by Riley (1957).

The approach described by Sverdrup (1953) is based on a comparison between the depth of the mixed layer and the depth at which light conditions (radiation and transparency) are sufficient to allow a net increase in the production of a water column. The latter depth is known as the "critical depth" and is defined as the depth at which the total production beneath a unit surface is equal to the total respiration. It follows that, if the critical depth is greater than the depth of mixing, a net increase in production can take place. Sverdrup (1953) determined a mathematical expression for the critical depth as follows:

$$\frac{D_{cr}}{1 - e^{-k_e D_{cr}}} = \frac{I_e}{I_c k_e} \qquad (1)$$

where D_{cr} is the critical depth in metres, k_e is the extinction coefficient (m^{-1}), I_e is the average energy which passes the sea surface per unit time and is available for photosynthesis, and I_c is the energy at the compensation depth.

[1]Received for publication August 6, 1965.

J. Fish. Res. Bd. Canada, 23(4), 1966.
Printed in Canada.

(The compensation depth is defined as the depth at which the energy intensity is such that production by photosynthesis balances destruction by respiration.) I_e and I_c have been expressed in langleys (ly) per hour, one langley being equal to one gram calorie per square centimetre.

Since the term $1-e^{-k_e D_{cr}}$ is little different from 1 the equation can be simplified to:

$$D_{cr} \doteqdot \frac{I_e}{I_c k_e} \tag{2}$$

In the following discussion the model described above was applied to the northeast Pacific Ocean. This area was arbitrarily defined as lying between 40 and 60°N lat from 160°W long to the coast of North America and thus practically corresponds to the eastern subarctic Pacific. Within this area special reference is made to Ocean Station P (50°N lat, 145°W long).

The conditions for a net increase in primary production described in this discussion were compared with the distribution of copepods at Ocean Station P and at several other points in the northeast Pacific. The latter data were employed here for two reasons. Firstly, 6 years of chlorophyll *a* data collected at Station P indicated that throughout the year very little change is observed in the standing stock of phytoplankton per cubic metre (Parsons, MS, 1965). There is good evidence that this absence of a marked change is due to intensive zooplankton grazing (McAllister et al., 1960), which must commence simultaneously with a net increase in the primary production during the spring. (Another example of this suppression of changes in the standing stock of phytoplankton per cubic metre is discussed by Heinrich (1962).) Thus, studies at Station P suggested that, in the eastern subarctic Pacific, changes in the standing stock of copepods (as herbivorous feeders) may be used to indicate a net increase in primary production during the spring.

The second reason for employing these data is that they represent the most geographically comprehensive data collected in this area during the spring. In contrast, C-14 primary production data are lacking except at Station P, where some seasonal changes have been reported and are discussed at the end of this presentation.

As far as available data permitted, results reported in this presentation were averaged over several years in order to obtain a general assessment of the occurrence of the spring bloom from which future anomalies can be observed.

METHODS

The critical depth was determined using expression 2 above. The extinction coefficient, k_e (m^{-1}), was calculated for maximum and minimum values for each month from Secchi disc data accumulated during 1957 through 1962 at Ocean Station P (Parsons, MS, 1965). These values are entered in Table

I as extinction coefficients for blue light as derived from the formula given by Poole and Atkins (1929):

$$k_e = \frac{1.7}{D},$$

where D is the Secchi disc depth in metres. For the remainder of the eastern subarctic Pacific, Utterback and Jorgensen's (1934) oceanic average extinction for the area, 0.073 m^{-1} at 4800 A, was employed. This value is representative of minimum values at Station P during the spring. Maximum extinctions during the spring at Station P also were included in calculations of the critical depth in the remainder of the northeast Pacific by using the maximum extinction for March of 0.13 m^{-1} (Table I).

The value used by Sverdrup (1953) for I_c, the energy at the compensation depth, was taken from Jenkin (1937) as being 0.13 ly/hr. This value was determined for a day length of 16 hr, which suggests that a slightly larger value might be considered for the spring when the day length is less. However, in the absence of sufficient information on the effect of light and dark periods on the compensation light intensity, the value of 0.13 ly/hr was employed

TABLE I. Estimations of the critical depth at Ocean Weather Station P (50°N, 145°W).

Month	Mean radiation[a] (*ly/hr*)	Mean radiation corrected for reflection (*ly/hr*)	PAR[b] (*ly/hr*)	Compensation light intensity (*ly/hr*)	Extinction coefficient		Critical depth (*m*)	
					k_{max} (m^{-1})	k_{min} (m^{-1})	Max	Min
Jan.	2.91	2.30	0.46	0.13	0.11	0.065	54	32
Feb.	4.73	4.11	0.82	0.13	0.12	0.068	93	53
Mar.	8.50	7.82	1.56	0.13	0.13	0.074	162	98
Apr.	11.2	10.6	2.12	0.13	0.15	0.081	200	108
May	15.3	14.7	2.94	0.13	0.18	0.089	253	126
June	15.3	14.7	2.94	0.13	0.20	0.095	237	113
July	14.4	13.8	2.76	0.13	0.23	0.10	211	92
Aug.	12.5	11.9	2.38	0.13	0.24	0.097	189	77
Sept.	10.3	9.6	1.92	0.13	0.23	0.089	165	64
Oct.	7.25	6.45	1.29	0.13	0.19	0.083	121	52
Nov.	3.71	3.15	0.63	0.13	0.15	0.076	62	32
Dec.	2.16	1.68	0.34	0.13	0.13	0.071	37	20

[a]Average radiation for each month determined from January 1960 to February 1964 (Monthly Radiation Summary, Meteorological Branch, Dept. Transport, Canada).
[b]Photosynthetically active radiation.

here for comparability with other authors (Marshall, 1958; Sverdrup, 1953; Cushing, 1962).

The average energy available for photosynthesis per unit time, I_e, was determined at Station P from the total solar radiation measured with an Epply pyrheliometer. These values, averaged from January 1960 to February 1964, were expressed as the mean hourly radiation and corrected for reflection losses by determining the mean sun altitude for each month (Sverdrup, 1953). The amount of energy available for photosynthesis was determined by reducing the total radiation by a factor of 0.2 to allow for absorption of nonphotosynthetic energy in the first metre of sea water (Sverdrup, 1953).

For the rest of the eastern subarctic Pacific, solar radiation estimates computed by the U.S. Bureau of Commercial Fisheries, San Diego (Marine Weather Observation Summary for the Pacific Ocean), were utilized. These data, which were available by 5° squares of latitude and longitude for the years 1962 through 1964, were averaged and expressed as the hourly photosynthetic radiation, corrected for cloud cover and for reflection, for each month.

Estimates of the depth of the mixed layer during February for the years 1947 through 1963 were taken from Giovando and Robinson (MS, 1965). For the areas 55–60°N, 150–160°W, and 40–45°N, 150–160°W, where no statistical estimation of the depth for the mixed layer has been made by these authors, an approximation of the mean mixed layer depth plus or minus one standard deviation was made from bathythermograph data (available at the Pacific Oceanographic Group, Nanaimo) for January to April during the years 1957 and 1962 through 1964. Data on the mixed layer depth obtained by Giovando and Robinson (MS, 1965) are represented in Fig. 1 for each month

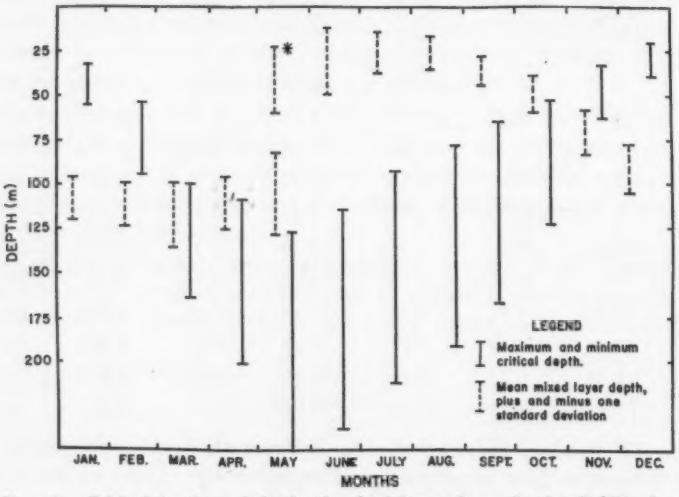

FIG. 1. Critical depths and the depths of mixing at Ocean Station P. *(During the years 1956–59 and 1962–63 the new seasonal thermocline was formed near the middle of May.)

of the year. In Fig. 2 are shown values of the mixed layer depth from north to south along lines of longitude, at 10° intervals, from 125 to 155°W. In this manner, estimates of the critical depth, which were made by 5° intervals of latitude and 10° intervals of longitude, can be compared with estimates of the mixed layer depth, which have been reported by areas composed of 5° intervals of latitude and 2° intervals of longitude (Giovando and Robinson, MS, 1965). In reporting the latter results, where two areas of latitude and longitude are adjacent (east and west) to a common line of longitude, the larger maximum and the smaller minimum values for the mixed layer depth are entered in Fig. 2.

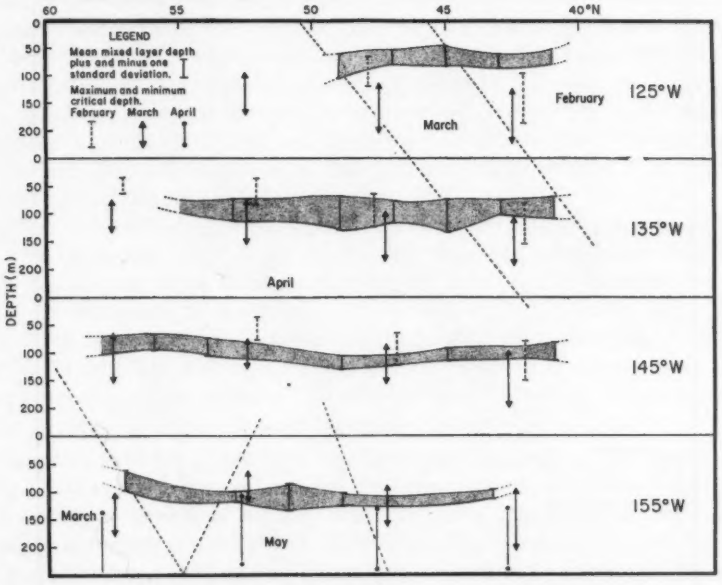

FIG. 2. Critical depths and the depths of mixing in the eastern subarctic Pacific Ocean, February–April. (Dotted lines separate areas in which conditions for a net increase in primary production became established in the same month.)

Maximum and minimum critical depths for March are also shown in Fig. 2 for each 5° of latitude and 10° of longitude. Critical depths for February are shown for 125 to 145°W and for April at 155°W. Other critical depth values for February and April were omitted only to clarify the figure and the following arguments would not be changed by their inclusion.

Copepod weights were determined from samples collected during April with a standard North Pacific net hauled vertically from 150 m to the surface. Total wet weight of copepods averaged by areas composed of 2° of latitude and 10° of longitude for the years 1962 and 1963 are shown in Fig. 3. Total copepod

wet weights at Station P averaged by month for the years 1956 through 1964 are reported as an inset to Fig. 3.

RESULTS AND DISCUSSION

From December to February the maximum critical depth at Station P was less than the minimum depth of mixing (Fig. 1). This would indicate that there could be no net increase in primary production at Station P during this period. On the other hand, from May through September the minimum critical depth was equal to or greater than the maximum depth of mixing, indicating that this is the principal period in which a net increase in primary production

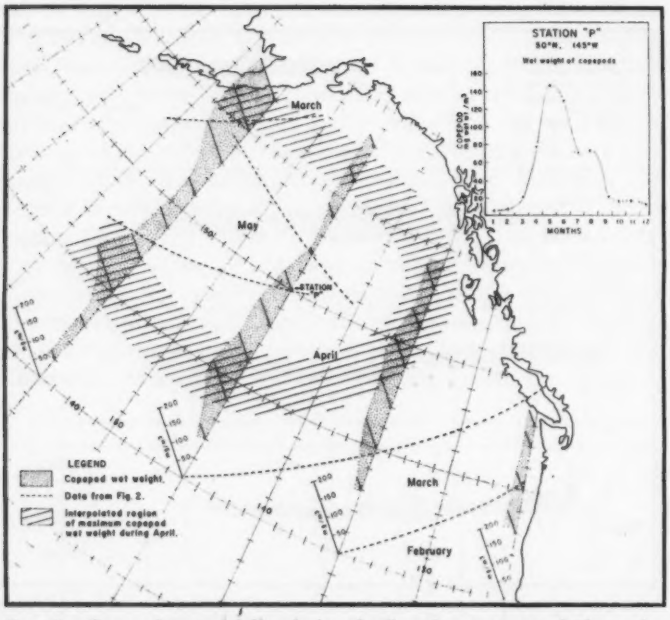

FIG. 3. Copepod wet weights during April and occurrence of the spring bloom in the eastern subarctic Pacific Ocean, February–May.

can occur. For 2 months during the spring, March and April, and 2 months during the fall, October and November, the conditions for a net increase in primary production were such as to indicate that some increase may take place under favourable conditions of radiation and stability.

In contrast to Fig. 1, it may be seen in Fig. 2 that, throughout a small area south of 45°N and east of 135°W, conditions for a net increase in primary production were possible during February. During March this area extended to 50°N at 125°W, and to about 45°N at 135°W. Another small area in which a net increase in primary production was possible developed during March in the vicinity of 55°N and 155°W. During April, critical depths exceeded the

depth of the mixed layer over the whole eastern subarctic region except between about 50° and 55°N at 155°W. Included also in this area (but not apparent from Fig. 2) should be the results for Station P (Fig. 1), for which the minimum critical depth was still above the maximum depth of mixing for April.

Dotted lines on Fig. 2, drawn to separate areas for which conditions for a net increase in primary production become established in the same month, were transposed to Fig. 3 where average copepod wet weights are shown for the month of April. An area of approximate copepod maxima was drawn as an interpolation of the results of copepod wet weights shown in this figure. Comparison of these results shows that maximum copepod weights were encountered in areas north and south of Station P in a semi-circle approximating the areas in which a net increase in primary production occurred during March to April. Minimum copepod weights were encountered in a central portion of the northeast Pacific between a wide area at 155°W and a narrower area extending east beyond Station P. This area of low copepod weight approximated the area in which conditions for a net increase in primary production were not firmly established until May. These results are further substantiated by the inset to Fig. 3 which shows that maximum copepod weights at Station P do not occur until the period May–June.

Other evidence supporting the description of the spring bloom discussed here can be found in primary production data for Station P (McAllister, 1962). These data show values of up to 1100 mg C/m^2/day during the period May–June compared with values of 200 or less mg C/m^2/day during March–April. Data reported by Stefansson and Richards (1964) show that in the area of 40–45°N and 125–130°W nitrate depletion starts in February and the nutrient becomes exhausted from the surface layers by May. These results are also in keeping with the sequence of events shown in Fig. 2 and 3.

Semina (1960) has described conditions for the onset of the spring bloom in the northwestern part of the Pacific Ocean, north of 55°N. Although the exact timing of events is not reported, the early onset of a spring bloom is indicated as far north as the Gulf of Anadyr. This is reported to be due to the high stability in the area caused by melting ice and river discharge. The former stabilizing influence is also reported in studies by Marshall (1958) in the Barents Sea. In this area, the onset of the spring bloom was reported to occur in April whereas, in Atlantic waters further south, sufficient stability was not achieved until June of the same year. In contrast to these results the early establishment of favourable conditions for a spring bloom in the northwestern corner of the Gulf of Alaska (Fig. 3) appears to be influenced more by solar radiation than by fresh water. Thus the mean total monthly radiation corrected for cloud cover and reflection during the period March–May in the region 55–60°N and 155°W is 300 ly/month compared with 207 ly/month in the region 45–55°N and 155°W (U.S. Bureau of Commercial Fisheries, San Diego, Marine Weather Observation Summary for the Pacific Ocean).

In conclusion, timing of the spring bloom in the northeast Pacific can be inferred from a knowledge of the critical depth and the mixed layer depth

during the 4-month period, February–May. However, the description given here applies to a protracted period in which other factors, such as currents, may also be significant in determining distribution of plankton. Thus while the general shape of the curves showing the distribution of copepods in Fig. 3 is in agreement with the description of the spring phytoplankton bloom, the absolute quantities of copepods at 155°W are greater than along other lines of longitude. An explanation for this type of variation is currently under investigation but it appears initially that one solution is to be found in separating growth rate and recruitment of the copepod stock. Preliminary results indicate that only the former is influenced by the timing of the spring bloom.

ACKNOWLEDGMENTS

The authors are grateful to the U.S. Bureau of Commercial Fisheries, San Diego, for making available the computed solar radiation data corrected for cloud cover and reflection in the northeast Pacific Ocean.

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Procedure for Analysis of Schooling Behavior[1]

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ABSTRACT

A method for the analysis of schooling behavior applicable to field and laboratory studies is presented. Three indices were developed to measure the behavior of a school, from motion pictures. Two of the indices are measures of the distance among individuals in a school, and the other is a measure of the differences in orientation among the individuals. A value for each index was calculated for each motion-picture frame analyzed. A coordinate reader and digitizer used to analyze films permitted the operator to record automatically on IBM cards the X and Y coordinates for the positions of the head and tail of each fish in a frame. A computer program was written to calculate the three indices from the coordinates, to convert the measurements to actual distance, and to make statistical comparisons.

Laboratory experiment on the influence of food deprivation on schooling behavior of juvenile jack mackerel, *Trachurus symmetricus* (Ayres) indicated: schools became compact immediately after feeding; the intervals among fish expanded during a deprivation period; and differences in orientation were related to the size of the individuals.

A procedure was needed to express quantitatively the behavior of schools of fish. The method needed to be suitable for both field and laboratory studies and had to be adapted to the schooling habits of fast-swimming pelagic fishes. This paper describes a procedure that has these attributes. To illustrate the functioning of this technique, laboratory data are presented on the effect of feeding and food deprivation on the behavior of schools of juveniles of jack mackerel, *Trachurus symmetricus* (Ayres).

Procedures previously used to quantify schooling behavior were designed for laboratory studies and would be difficult to appy in the field. They include: ranked estimates of the degree of aggregation (Bowen, 1931); measurements from photographs of the distance between two parallel fish in a school (Breder, 1954); density measurements based on visual approximation of the position of fish against vertical and horizontal grids (Keenleyside, 1955); calculations of swimming speeds from cinematographic data (Steven, 1959); and the perimeter of the smallest polygon which encloses a school (Williams, 1964). John (1964) developed an index of schooling based on the assignment of a rank to each possible horizontal grouping of six fish. If only two of the fish were near each other, the value 1 was used; if all fish were together, the value 10 was used; and intermediate groupings were assigned ranks between 1 and 10.

[1]Received for publication October 25, 1965.

J. Fish. Res. Bd. Canada, 23(4), 1966.
Printed in Canada.

No consensus exists on the essential characteristics of a school. Breder and Halpern (1946) considered the term to include aggregations of fish in which the spacing and orientation of the individuals are uniform. Keenleyside (1955) used a broader definition. He considered a school to be "an aggregation formed when one fish reacts to one or more other fish by staying near them." The jack mackerel is a schooling fish by either definition.

METHODS

Apparatus

Experiments were conducted in a light-tight chamber, 2.4 m wide, 3 m long, and 2.4 m high. The inside walls, ceiling, and all structures inside the chamber were painted flat white. A laminated plywood tank was constructed with the top in the same proportions as a 16-mm motion-picture frame. The tank was 141 cm long, 110 cm wide, and 27 cm deep. The inside of the tank was coated with white-pigmented resin and two black cross lines, for aligning the camera, were drawn on the bottom. The water in the tank was 18 cm deep and was recirculated.

A 16-mm movie camera was mounted inside a light-tight hatch on the outside of the chamber, 71 cm above the tank. The lens of the camera projected through a port, 11 cm in diameter, in the ceiling of the chamber directly over the center of the tank. The camera was driven by a synchronous motor and controlled by an interval timer.

The chamber was illuminated by eight 100-w, 2850° Kelvin, clear tungsten lamps spaced at equal intervals around the perimeter of the tank. The tops of the lamps were 2 cm below the upper edge of the tank wall. Since the tank walls were opaque, the only light entering the water was that reflected from the sides and ceiling of the chamber. The light in the tank was distributed relatively uniformly.

An electric clock with numerated cams for hours, minutes, and seconds, mounted on the outside edge of the tank appeared in every frame of the film. All experiments were conducted in a temporary laboratory that had a closed seawater system. A thermográph recorded the water temperature, which was 22 ± 2 C. Six juvenile jack mackerel were used in each experimental group — the same number used in certain other studies of schooling behavior (Keenleyside, 1955; John, 1964).

Three Indices of Schooling

Three indices were developed to measure schooling behavior from time-lapse photographs of schools in the horizontal plane. Two — mean separation distance and mean distance to the nearest neighbor — are measurements of the compactness of the school, and one — mean angular deviation — is a measure of the differences in orientation among individuals in the school. All measurements are in millimeters. Mean distance to the nearest neighbor is a

modification of a statistic used in plant ecology studies (Clark and Evans, 1954); the other two indices are new.

The *mean separation distance* is the average of the horizontal distances separating each fish from every other fish in the school. The distance from the head of one fish to the head of every other fish in the school is measured. These measurements are made for each fish in the school, except no measurement is duplicated. Thus, for a school of six fish, the mean of 15 measurements is designated the mean separation distance.

Mean distance to the nearest neighbor is the average of the distances from each fish in a school to its nearest neighbor. The measurement is made between the two closest points on the midline of the bodies of the two fish, regardless of their orientation. The same measurement is used twice if two individuals are closer to each other than to any other individual in the school.

The *mean angular deviation* is a measure of the differences in orientation among fish within a school. To calculate the index, the heading of each fish, in degrees, is determined. Then the resultant direction of the school is computed by assigning each fish a value of one and adding the headings vectorially. The number of degrees each fish deviates from the resultant direction of the school is determined and a mean calculated. This value is the mean angular deviation.

The values for mean angular deviation ranged from near 0 (for schools of uniformly oriented fish) to about 90° (for groups composed of randomly oriented individuals).

The three indices were calculated for each photograph of a school.

Films were analyzed by use of a coordinate reader and digitizer[2] to decrease data-processing time. To analyze a single photograph of six fish by hand with single-frame analyst projector required 30 min, but less than 30 sec were needed when a coordinate reader was used. The reader operated as follows: film was projected on a ground-glass screen; the operator, by turning two controls, moved an X and Y crossline across the glass screen; and the position of each coordinate was recorded on an IBM card by depressing a foot pedal. In this manner, the X and Y coordinates for the tip of the snout and the tail of each fish in a frame were recorded. A computer program[3] was written to calculate, from the coordinates, the three indices for each frame, to convert the reader units into the actual distance in millimeters, and to make statistical comparisons of groups of 25 indices (Mann-Whitney U test — Siegel, 1956).

One unit on the screen equaled 0.37 mm in the tank. The coordinates for the position of the snout and tail of the fish were measured with a precision of ± 4 reader units. Thus, the actual distance between fish was measured with a precision of ± 1.5 mm.

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[2]These are semi-automatic record-reading machines designed to analyze data recorded as images or traces on transparent, translucent, or opaque film or paper. Several models are available from manufacturers.

[3]Available in Fortran-63 program decks. Copies may be obtained from the U.S. Bureau of Commercial Fisheries Tuna Resources Laboratory, La Jolla, California.

Analysis of a Continuous Film

Groups of juvenile jack mackerel were almost continually in motion. The swimming speed of a group was not constant but varied from 7 to 15 cm/sec. The schools did not mill or show any habitual sequence of maneuvers. Groups appeared to make random passages in all areas of the tank with equal frequency.

Each frame of a continuous sequence was analyzed to determine the sensitivity of the three indices to minor changes in the dynamics of the school. Figure 1 shows the changes in each index for a school of six jack mackerel during a 100-frame sequence taken at a speed of 12 frames/sec. During this period, the school executed three turns; the first was the sharpest and the last

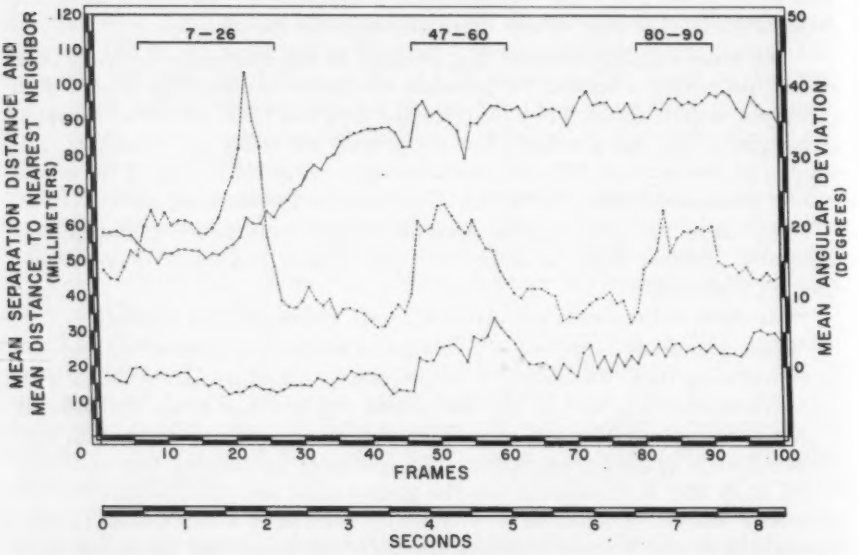

FIG. 1. Mean separation distances (upper, solid line), mean distance to nearest neighbor (lower, solid line), and mean angular deviation (middle, broken line) of a school of six jack mackerel for 100 continuous frames taken at 12 frames/sec. Brackets indicate the three major oscillations in angular deviation.

the most gradual (Fig. 2). The three peaks in mean angular deviation shown in Fig. 1 correspond with the three turns; the more acute the turn, the greater was the oscillation in the mean angular deviation.

The school was relatively compact prior to the first turn but lengthened after turning; the fish became arranged in an overlaping file 4–5 fish long. During the second turn the school shortened and widened; the fish became distributed in 2 or 3 ranks of 1–3 fish each. The school retained this form during the remainder of the sequence. Both indices of spacing became higher in response to this general decrease in compactness. As the school elongated

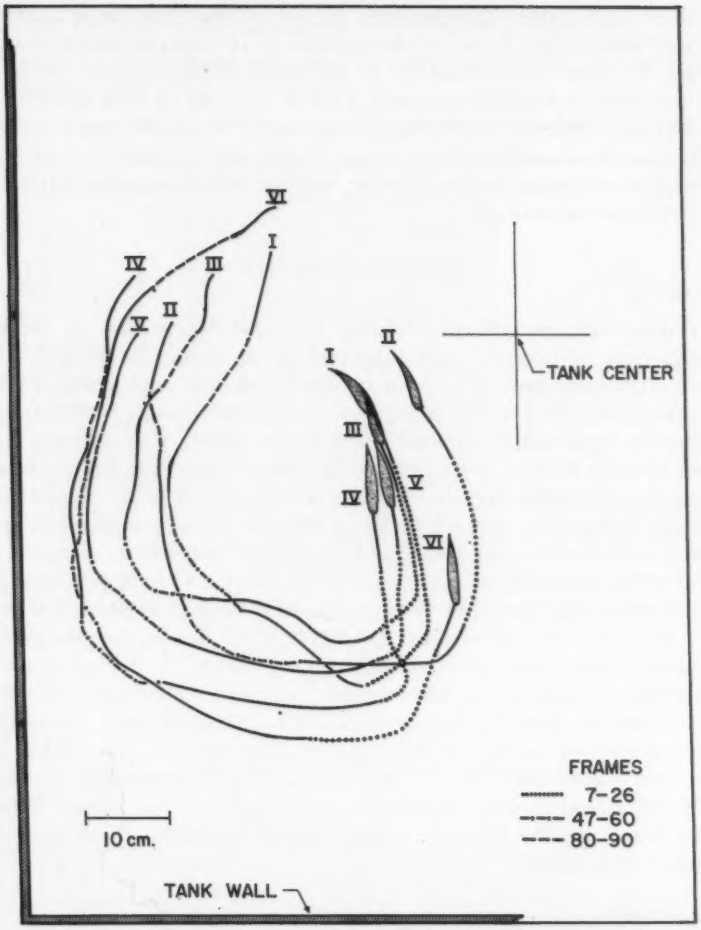

FIG. 2. Movements of the school of six jack mackerel during the 100-frame sequence shown in Fig. 1. Positions of the fish in the first frame are drawn. Subsequent positions are indicated by lines. The three groups of broken lines indicate the three major oscillations in angular deviation. (Roman numerals identify individual fish.) Movements were charted by projecting each of the 100 frames in sequence and plotting the position of each fish in every frame.

(frames 26–47) separation distance increased rapidly; distance to nearest neighbor, on the other hand, did not change. At the start of the second turn (frame 47) distance to the nearest neighbor increased sharply but separation distance, already high, increased only slightly.

Analysis of continuous film demonstrated that the indices were sensitive to minor changes in the activities of a school. If the period under study was

minutes or hours rather than seconds, analysis of every frame in a continuous series was impractical. If single photographs were taken at intervals of 1 min or longer, an observation could fall at any point in the graph shown in Fig. 1. Thus, the angular deviation for such a point could be as high as 43° or as low as 6°. Similar variability would be encountered in the mean separation distance and distance to nearest neighbor. Thus, if intermittent observations are used, considerable oscillation can be expected between points and only major changes in behavior can be evaluated.

FOOD DEPRIVATION

Procedures

To determine the effect of feeding and food deprivation on the spacing and alignment of juvenile jack mackerel, three groups of six fish of three different sizes were used. The mean standard length of each group was: group A, 69 mm; group B, 84 mm; and group C, 106 mm. The fish were fed frozen brine shrimp by lowering the food through the camera port. Groups A and B received 10 g per feeding, and group C, 15 g per feeding. The size of the rations was determined during previous feeding periods and was the amount of food required by each group to reach satiation. Schools were photographed at 1-min intervals for 54 hr. The fish were fed twice during the period: once at the end of a 24-hr deprivation period, and again at the end of a 48-hr deprivation period.

Each group was placed in the experimental tank at 1500 hr, 2 days prior to the initiation of the experiment. At 1100 hr the following day the group was fed. Filming began 22 hr later, at 0900 hr; 2 hr later the fish were fed. They were not fed again for 48 hr; photography was terminated 4 hr after the last feeding. From the time the fish were placed in the tank until filming began, the fish experienced normal daylight, as the chamber was illuminated by the sunlight that passed through the open door. When photography started, the lights in the chamber were turned on, and the door was closed. The door remained closed and the fish were under constant illumination until the experiment ended 54 hr later.

Data Analysis

In each experiment every frame was analyzed for the 50 min before each feeding and for the 150 min after food was introduced. The 200 values for each of the indices were grouped separately, for statistical comparison, into 8 sets of 25 values each. Sets 1 and 2 occurred before feeding and sets 3 through 8 after food was added. This arrangement allowed a detailed analysis of the change in schooling behavior associated with feeding. The data are presented in their original form for group C; for groups A and B only the results of the statistical comparisons are shown.

The Mann-Whitney *U* test was used to make all statistical comparisons. Although this nonparametric test functioned well in this application, its selection was more or less arbitrary. Undoubtedly, other tests could be used with equal effectiveness.

Separation Distance and Distance to Nearest Neighbor

Schools of jack mackerel were more compact during and after feeding than before they were fed (Fig. 3–6; Table I).

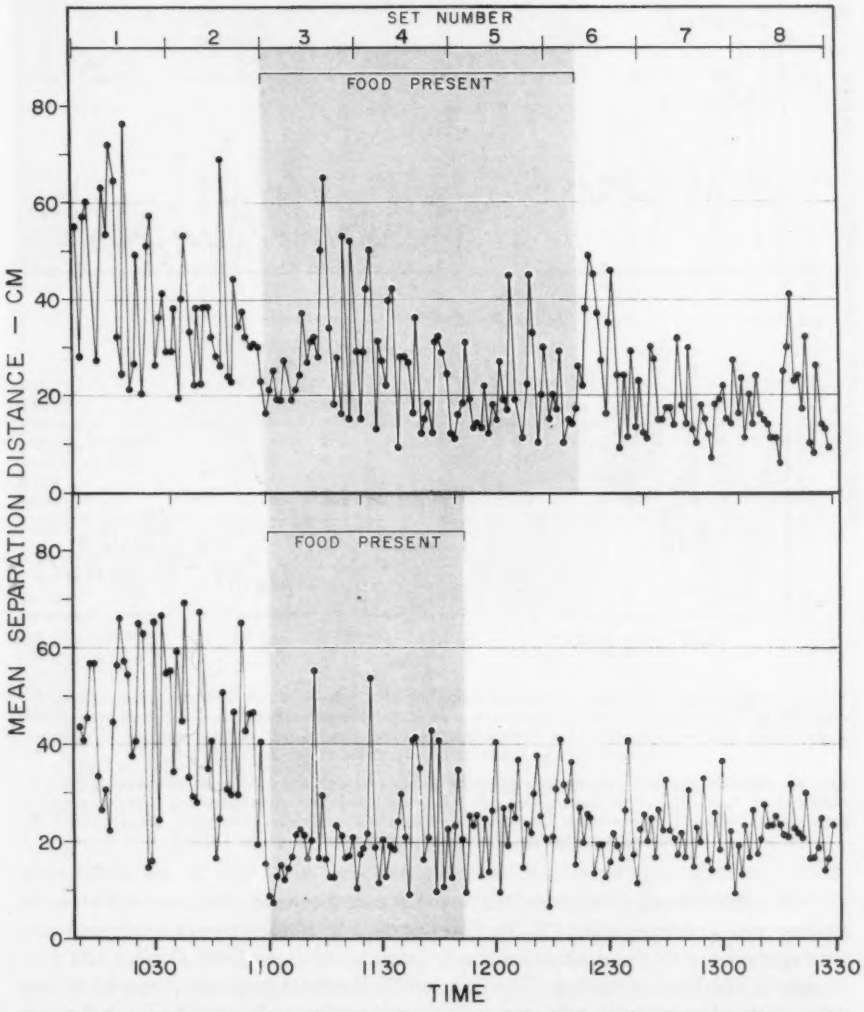

Fig. 3. *Top.* Mean separation distances of a school of six jack mackerel (group C) recorded at 1-min intervals during and after a 24 hr food-deprivation period. Shaded area represents period food was present. One-minute intervals are divided into eight groups of 25 observations each for the statistical comparison shown in Table I.

Fig. 4. *Bottom.* Mean separation distances of a school of six jack mackerel (group C) recorded at 1-min intervals during and after a 48-hr food-deprivation period. Shaded area represents period food was present. One-minute intervals are divided into eight groups of 25 observations each for the statistical comparison shown in Table I.

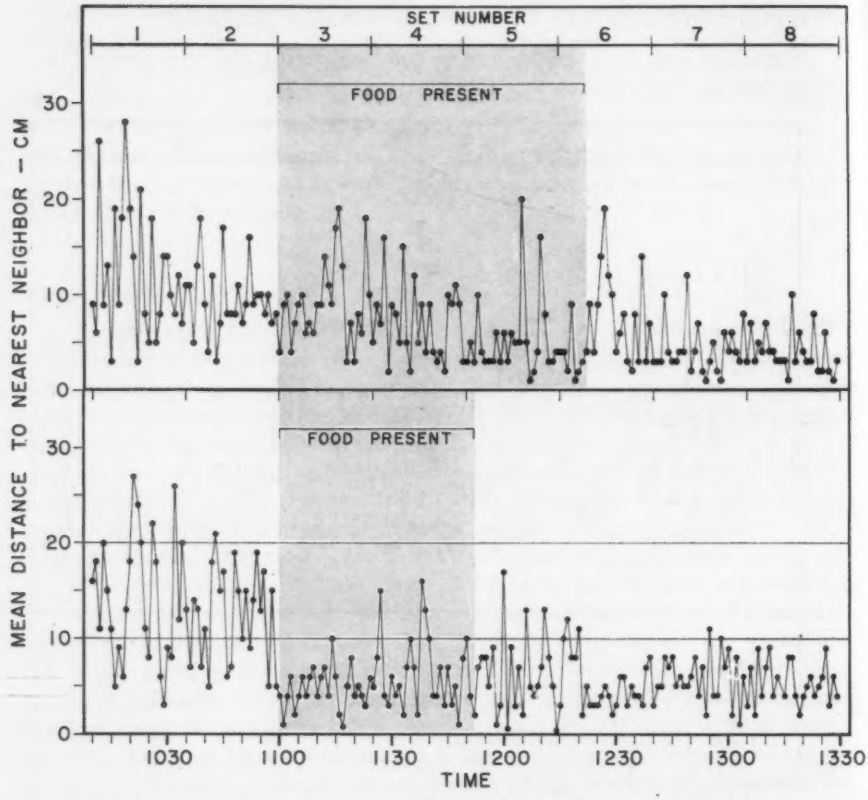

FIG. 5. *Top.* Mean distances to nearest neighbor of a school of six jack mackerel (group C) recorded at 1-min intervals during and after a 24-hr food-deprivation period. Shaded area represents period food was present. One-minute intervals are divided into eight groups of 25 observations each for the statistical comparison shown in Table I.

FIG. 6. *Bottom.* Mean distances to nearest neighbor of a school of six jack mackerel (group C) recorded at 1-min intervals during and after a 48-hr food-deprivation period. Shaded area represents period food was present. One-minute intervals are divided into eight groups of 25 observations each for the statistical comparison shown in Table I.

The difference in mean separation distance between the two sets prior to feeding was not significant (Table I, sets 1 and 2). Differences were significant between set 1 and those after food was introduced (sets 3–8). During the first 25 min of the feeding period (Fig. 3–6, set 3) the food was concentrated in one area and frequently the fish were aggregated near the food. The food became dispersed thereafter and the spacing of the fish within the school was more directly a reflection of their behavior rather than the distribution of the food.

In group A, after 24 hr of deprivation, not all of the sets of observations after the introduction of food were different from set 1; after the 48-hr deprivation period, all of these sets (3–8) were different from set 1. Greater differences

TABLE I. z values[a] obtained from statistical comparisons of three indices of schooling behavior for three jack mackerel schools; set 1 (before feeding) is compared to set 2 (before feeding) and sets 3–8 (during and after feeding). (Values for which $p > 0.05$ are indicated by ns.)

Relation to time of feeding	Values of set 1 compared with those of set:	Fed after 24 hr deprivation			Fed after 48 hr deprivation		
		A	B	C	A	B	C
		Separation distance					
Before	2	1.43 ns.	0.01 ns.	1.91 ns.	0.92 ns.	0.46 ns.	1.19 ns.
During	3	5.77	5.25	3.11	4.96	5.85	5.05
During	4	2.59	3.75	3.41	3.29	4.26	4.36
During or after[b]	5	1.29 ns.	4.22	4.74	4.59	4.41	4.47
During or after	6	2.45	3.09	4.03	5.56	3.87	4.47
After	7	1.29 ns.	3.68	5.27	5.09	4.34	4.80
After	8	0.55 ns.	3.29	5.17	5.15	4.55	4.76
		Distance to nearest neighbor					
Before	2	1.85 ns.	2.03	1.25 ns.	0.84 ns.	0.94 ns.	0.88 ns.
During	3	5.93	5.00	1.48 ns.	4.32	5.19	5.15
During	4	3.52	4.10	2.76	1.93 ns.	3.81	4.24
During or after[b]	5	2.20	2.76	4.34	3.54	3.48	4.20
During or after	6	3.29	2.34	3.15	5.42	3.44	4.78
After	7	1.33 ns.	3.35	4.78	5.09	3.52	4.61
After	8	1.70 ns.	2.20	5.00	3.95	3.68	4.92
		Angular deviation					
Before	2	0.28 ns.	0.36 ns.	1.29 ns.	0.03 ns.	0.40 ns.	0.03 ns.
During	3	2.32	2.44	3.93	2.28	1.70 ns.	1.31 ns.
During	4	0.57 ns.	2.40	1.41 ns.	0.69 ns.	2.82	0.55 ns.
During or after[b]	5	1.14 ns.	2.67	1.06 ns.	4.34	1.48 ns.	1.66 ns.
During or after	6	0.69 ns.	2.98	0.15 ns.	4.86	2.36	2.28
After	7	1.15 ns.	2.82	0.20 ns.	4.90	1.19 ns.	3.72
After	8	0.24 ns.	2.32	1.25 ns.	5.07	1.29 ns.	3.62

[a]Test criterion of Mann-Whitney U test.
[b]Time required for each school to consume all food varied. For all groups, the food was consumed by the end of set 6.

were obtained in the 48-hr test because the fish were spaced more widely before they were fed. The mean separation distance immediately after feeding was the same regardless of the length of the preceding deprivation period.

The schools became more compact during the time that would have been the first hours of darkness if the fish had not been kept under constant illumination (Fig. 7). A possible explanation is that prior to the experiment the fish may have responded to lower levels of illumination by swimming closer together, and this behavior persisted under constant illumination.

The times at which the fish were fed are represented by the second and penultimate points in Fig. 7. These points were lower than the preceding points, as would be expected from the analysis of the data presented above.

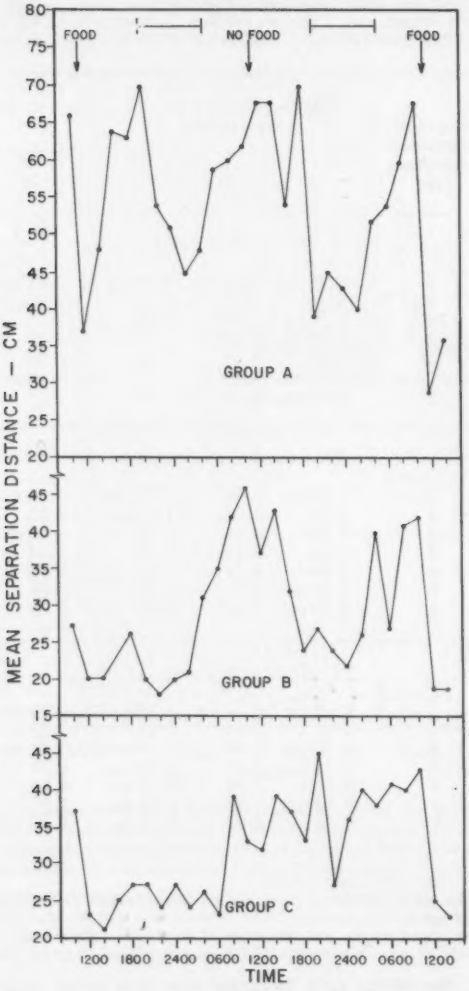

FIG. 7. Mean separation distances for three groups of six jack mackerel during a 54-hr period under constant illumination. Each point represents 2 hr and is an average of 12 individually calculated mean separation distances. Time scale indicates midpoint of each 2-hr period. Horizontal bars near top of upper panel represent the period each day that fish were in the dark prior to filming. Fish were fed at 1100 hr on the first and third day of the experiment.

Mean distance to nearest neighbor (Fig. 5, 6; Table I) showed the same changes during and after feeding as did mean separation distance. Distance to nearest neighbor also decreased during the first hours of darkness as described

above for separation distance. A graph of these data was not included because of the close resemblance to that of separation distance.

Angular Deviation

The mean angular deviation of schools changed during feeding. In the initial stage of feeding (set 3) the fish fed continually and independently. This behavior caused an increase in the mean angular deviation (Fig. 8; Table I). After approximately 25 min the school re-formed. Thereafter, fish left the school briefly to feed until all food was consumed. A fish that left the school, executed a short charge, captured a brine shrimp, and returned.

Toward the end of the 48-hr deprivation period, groups B and C showed as great differences in orientation as they did during the initial stage of feeding. The angular deviation for these two groups did not increase during the first 25 min of the second feeding period but remained at the same level (Fig. 8, 9).

The three groups (Fig. 10) showed different levels of angular deviation during the 54-hr experimental period; the angular deviation of the school was related to the size of the fish. Group A, composed of the smallest fish, was the least oriented school; group C, composed of the largest fish, showed the least differences in orientation. Group B was intermediate in both fish length and orientation of the school. Tests based on all points shown in Fig. 10 demonstrated that the differences between each group and every other group were significant at the 0.001 probability level or lower.

The smaller differences in orientation of the larger juveniles probably was not an artifact related to the size of the tank. If the size of the tank had influenced the results, the larger fish would have been more disorganized because, owing to their size, they were forced to turn more frequently than were smaller fish.

Differences in behavior between the fish composing group A and the larger fish of groups B and C were noted at the time the fish were captured. All fish were caught at night beneath a lamp. Fish of the size of those in group A swam beneath the light individually; larger individuals, as those in groups B and C, swam beneath the light in a school. Nevertheless, fish of group A schooled in the laboratory although they were usually more widely spaced (Fig. 7) and showed greater differences in orientation than did the larger fish.

Evaluation of Indices

Mean separation distance and mean distance to nearest neighbor are measurements of the relative compactness of schools but the information they provide is not identical. Separation distance is an average of all spatial relationships among fish in a school, whereas distance to nearest neighbor is an average of only the one relationship. Distance to nearest neighbor cannot distinguish between a school whose individuals are congregated in one spot from a school consisting of scattered groupings of individuals (Clark and Evans, 1954). A plot of separation distance against distance to nearest neighbor shows

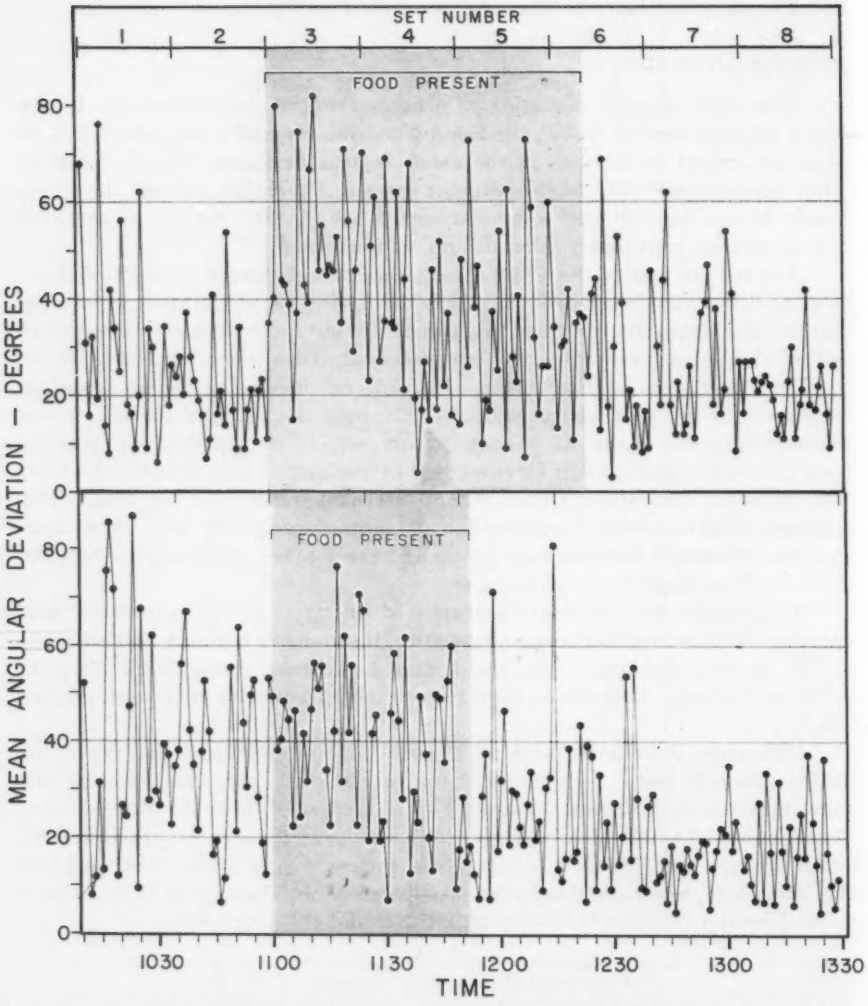

FIG. 8. *Top.* Mean angular deviations of a school of six jack mackerel (group C) recorded at 1-min intervals during and after a 24-hr food-deprivation period. Shaded area represents period food was present. One-minute intervals are divided into eight groups of 25 observations each for the statistical comparison shown in Table I.

FIG. 9. *Bottom.* Mean angular deviations of a school of six jack mackerel (group C) recorded at 1-min intervals during and after a 48-hr food-deprivation period. Shaded area represents period food was present. One-minute intervals are divided into eight groups of 25 observations each for the statistical comparison shown in Table I.

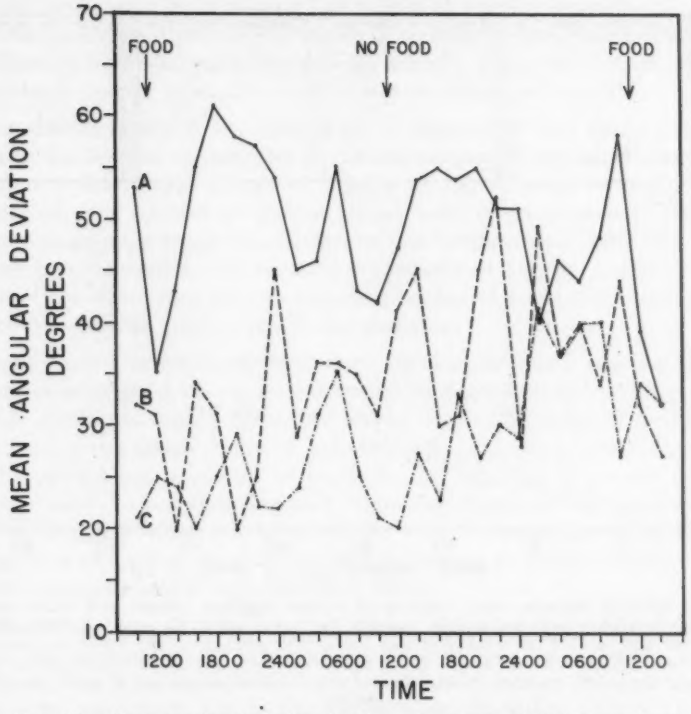

FIG. 10. Mean angular deviations for three size groups of six jack mackerel during a 54-hr period under constant illumination. Each point represents 2 hr and is an average of 12 individually calculated mean angular deviations. Time scale indicates midpoint of each 2-hr period. Mean length of fish in each group was: A, 69 mm; B, 84 mm; and C, 106 mm standard length.

that the relation between the two indices is variable (Fig. 11). Most of the variability is caused by the inclusion in separation distance of measurements of the distances between groups within a school (Fig. 11, B and C). The relationship between the two indices was more variable before than after feeding because widely spaced schools more frequently form subunits than do compact schools. Since an increase in density of a school can include both the disappearance of subunits and a decrease in distances among neighbors, separation distance can be a more powerful tool for detecting changes in compactness. Separation distance, however, is affected by both the size and the number of individuals in a school; distance to nearest neighbor, on the other hand, is unaffected by these variables. In the measurements of distance to nearest neighbor, a slight bias exists in the present data which is related to size because the distances are measured between the midline of the body rather than from the outer edge. In the present application this difference seems unimportant. For comparison of fishes that differ greatly in size, as adults and juveniles,

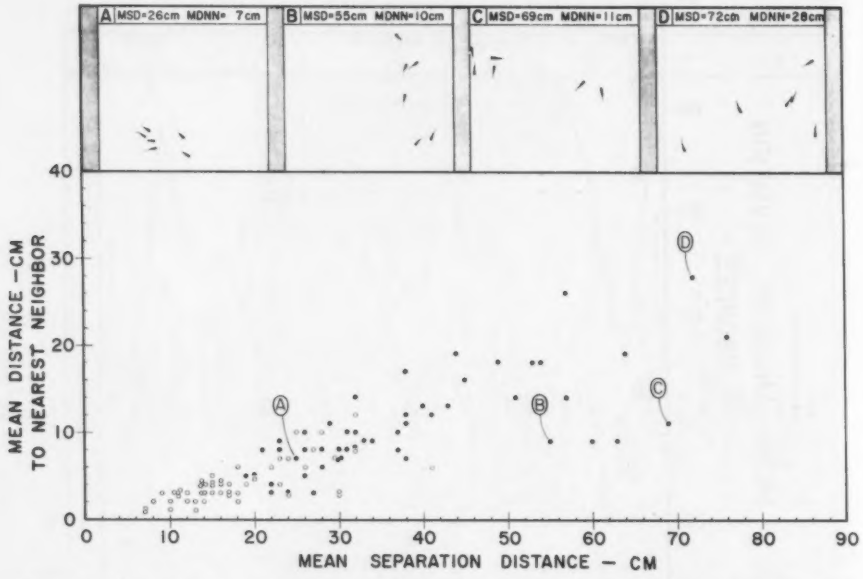

FIG. 11. Relation between mean distance to nearest neighbor (MDNN) and mean separation distance (MSD). Data were taken from the first feeding of group C: sets 1–2 (before feeding), solid circles; and sets 7–8 (after feeding) open circles. The positions of the fish in the photographs that produced points A–D are shown above figure. Points A and D are representative of those which showed a nearly constant relationship between the two indices, and B and C are divergent points.

this bias could be corrected by adjusting the measurements for average body width.

In summary, separation distance and distance to nearest neighbor have complementary functions because comparisons of them yield information on the formation of subgroups. Each has a specific function, however; for a relative measure of changes in spacing of an entire school, separation distance is preferable, but for a comparison of samples containing different numbers of individuals or individuals that differ greatly in size, distance to nearest neighbor must be used.

The parallel alignment of individuals in a school has either been excluded from measurements of schooling (Keenleyside, 1955) or it has been included in the method of measuring dispersion or spacing (Breder, 1954; John, 1964). Mean angular deviation permits an independent analysis of the alignment of individuals in a school. Unlike separation distance, mean angular deviation is independent of the size of individuals. It is, however, somewhat affected by the number of individuals in a sample. The mode of a frequency distribution of 1 million values of mean angular deviation based on six randomly determined angles per sample was 69°, but that based on 12 angles per sample was 75°.

Thus, the probability of obtaining low values of angular deviation from random data is somewhat greater if a school of 6 rather than 12 fish is used. Since this difference becomes negligible in larger schools, adjustments probably need be considered only in laboratory studies where school size is small.

For schools in which the uniformity of orientation is in doubt it may be desired to test for the randomness of the orientation. A frequency distribution of randomly determined values of angular deviation must be computed for this purpose. The number of random angles used to compute each mean angular deviation must equal the number of fish in the school. This distribution can then be compared to the data by a goodness of fit test; a significant departure of the data from the random distribution of angular deviation would indicate that the fish were oriented nonrandomly.

Data from this study showed that angular deviation was more useful than either separation distance or distance to nearest neighbor for recording behavior of schools during turns and during the initial stage of feeding, and for measuring the effect of size of individuals on schooling behavior. Angular deviation would not be useful to quantify the behavior of schools in which the fish usually do not show parallel alignment. It might help a comparative study of schooling behavior, however, because a continuum of forms exist, ranging from those that show no parallel alignment to the practically uniform schools of clupeids and scombroids (Atz, 1953).

Swimming-speed determinations (Steven, 1959) would be a useful supplement to the information provided by the three indices. Undoubtedly, both the distance among fish and orientation of the individuals in the school are influenced by swimming speed. Compared with the other indices, however, swimming speed requires much more time to calculate and also has the disadvantage that a continuous series of frames must be taken.

The methods described were designed for the analysis of the movements of fish in the horizontal plane only. There is no reason, however, that this approach could not be used to analyze vertical photographs of schools. The horizontal component was selected for this study because of the apparent importance of lateral orientation and because I plan to use this method for the analysis of films of schools taken from the air.

ACKNOWLEDGMENTS

The coordinate reader and digitizer was made available through the courtesy of the U.S. Navy Electronics Laboratory, San Diego. Warren A. Simmons of the computer section instructed in the operation of the reader. Richard R. Whitney, Tuna Resources Laboratory, provided editorial and statistical advice; C. M. Breder, Jr., Kenneth John, and Garth I. Murphy read the manuscript and offered helpful suggestions. Jack Mahan assisted in the study in numerous ways.

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Preparation and Properties of Trout Myosin[1]

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ABSTRACT

Myosin from the white, dorsal muscle of trout was isolated by a modification of the method of Mackie and Connell (1964). This myosin was homogeneous in the ultracentrifuge at 5 C. The corrected sedimentation coefficient, $S^{\circ}_{20,w} = 6.50$, was similar to those for cod and rabbit myosin. The isolated protein had two enzymatic activities, an adenosine triphosphatase (ATPase) and an acetylcholinesterase. The rate of inactivation of the acetylcholinesterase activity at 45 C was approximately equal to the rate of inactivation of the ATPase activity at 25 C. Inactivation of the ATPase was a first-order process with respect to time and 0.6th order with respect to protein concentration. The energy of activation was 46 kcal mole^{-1} at *p*H 6.8, lower than values reported for rabbit myosin. Using the loss of enzyme activity as a measure of denaturation, trout myosin was about 23× more stable than cod myosin, but lost its activity about 25× more rapidly than rabbit myosin. Some of the mechanisms active in denaturing myosin are briefly discussed.

INTRODUCTION

Development of undesirable changes during handling and storage of fish has been attributed to denaturation of the myofibrillar proteins. It seems desirable, therefore, to obtain a model system and to study interactions between it and other cell constituents as well as the effect of postmortem conditions which are likely to change the properties of myosin and related structural proteins. A better knowledge of the properties of myosin may eventually lead to an understanding of reactions leading to the poorly defined condition of "protein denaturation" as used in fish technology.

Procedures for the extraction of ultracentrifugally homogeneous skeletal and cardiac myosin from different mammalian species have been established (Johnson and Rowe, 1960; Mueller et al., 1964). Chromatography of rabbit myosin and removal of nucleotide contaminants have been carried out on DEAE-cellulose and DEAE-Sephadex columns (Asai, 1963). Extraction procedures for the preparation of fish myosins were pioneered by G. Hamoir, University of Liège, and by J. J. Connell, Torry Research Station. Methods of isolation and properties of purified, unaggregated cod myosin have been described (Mackie and Connell, 1964). Chromatography of myosins from yellowfin and albacore tuna, and from chicken and rabbit muscle was carried out on DEAE-Sephadex A-50 columns (Richards et al., 1965).

In this study, the extraction and some of the properties of myosin from the white dorsal muscle of trout (*Salmo gairdneri*) are reported.

[1]Received for publication October 12, 1965.

J. Fish. Res. Bd. Canada, 23(4), 1966.
Printed in Canada.

EXPERIMENTAL

Preparation of Myosin

The extraction procedure for the isolation of trout myosin was essentially that of Mackie and Connell (1964). Modifications were made in the type of buffer used and by treating the extract at one step in the procedure with 15% dimethylsulfoxide (DMSO). Lowering the ionic strength to $\mu = 0.23$ did not seem to improve the purity of the myosin and was therefore omitted in most preparations.

All operations were carried out in a cold room (0 C) with prechilled equipment and solutions. *Step I.* A freshly killed trout was filleted and 25 g of white skeletal muscle were homogenized twice with 280 ml of 0.0050 M KH_2PO_4–0.0150 M $NaHPO_4$ buffer (ionic strength $\mu = 0.05$, *p*H 7.4). Homogenization was carried out in a Waring Blendor, closed by a baffle plate. The low ionic strength soluble material was removed by filtration on a Buchner funnel and Whatman No. 31 filter paper. *Step II.* The insoluble residue was homogenized in 280 ml of 15% DMSO. The resulting suspension was centrifuged at about 7000 *g* for 10 min. *Step III.* The supernate was decanted and the insoluble material was washed further, once with the above phosphate buffer and finally with a histidine buffer ($\mu = 0.05$, *p*H 6.8). *Step IV.* The insoluble material remaining (16.4 g) was then extracted with twice its weight (32.8 ml) of 0.7 M KCl – 0.05 M histidine buffer *p*H 6.8. At the beginning of the extraction, 0.15 ml of 0.2 M $Na_4P_2O_7$ and 0.015 ml 2.0 M $MgCl_2$ per gram of insoluble material were also added. After 15–20 min the suspension was centrifuged at 4000 *g* for 15 min. *Step V.* The supernate was carefully decanted, weighed (54.1 g), and diluted with four times its weight (216.4 ml) of ice-cold, glass-distilled water. The cloudy suspension was centrifuged at once at 8000 *g* for 10 min. *Step VI.* The supernate was discarded and the precipitate was weighed (38.60 g) and dissolved in one-third its weight (12.86 ml) of 0.9 M KCl – 0.1 M histidine buffer *p*H 6.8. After centrifugation at 27,000 *g* for 10–15 min the supernatant solution contained about 1% protein. No notable precipitate formed when the ionic strength was reduced to $\mu = 0.23$.

The reason for choosing DMSO treatment was to remove compounds such as lipids, nucleic acids, and nucleotides found in association with myosin. How well DMSO accomplished this was not extensively investigated. A striking fact during the extraction procedure was, however, that the preparation yielded a clearer solution, after the precipitate from the dilution step (step VI) was taken to higher ionic strength. Without solvent treatment, the solution at this point was still turbid.

Ultracentrifugation

Sedimentation velocity measurements were made in a Spinco Model E ultracentrifuge. The results were calculated as described by Svedberg and Pedersen (1940) and by Schachman (1957). Myosin samples used in the centrifugation experiments were never older than 48 hr from the time the fish had been killed.

FIG. 1. Sedimentation patterns of trout myosin at 5 C. Sedimentation from right to left, 98, 106, 114, 122, 130, and 138 min after the rotor reached full speed (52,640 rpm); bar angle 60°; solvent, KCl-histidine, $\mu = 0.50$, pH 6.8; protein concentration 0.25%.

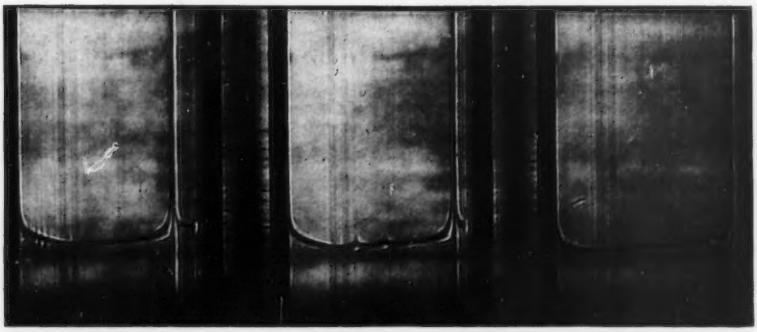

FIG. 2. Sedimentation patterns of trout myosin at 20 C, demonstrating the aggregates which have formed in a 0.40% solution of trout myosin. The solution had been kept at room temperature for 5 min before centrifugation. Sedimentation from right to left, 0, 16, and 32 min after the rotor reached full speed (52,640 rpm); solvent, KCl-histidine, $\mu = 0.50$, pH 6.8.

Protein

Measurement of the spectral properties of myosin in the ultraviolet region were made with a Beckman D.B. recording spectrophotometer. Protein nitrogen was determined by a micro-Kjeldahl method. The results were used to standardize the biuret procedure as described by Snow (1950). All optical density measurements were made against a blank containing the buffer used. Protein concentrations were calculated by assuming a nitrogen content of 16%.

ATPase Activity

All reagents used in the ATPase determinations were made up to an ionic strength $\mu = 0.5$, using KCl–histidine buffer. The reaction mixture for the determination of myosin ATPase was made up as follows: 0.1 ml of 0.1 M $CaCl_2$ and 1.6 ml of 0.45 M KCl – 0.05 M in histidine (pH 6.8) were mixed in a test tube. Enzyme solution 0.2 ml, containing from 0.250 to 0.625% protein ($\mu = 0.5$), was added to the above mixture. The tubes were placed in a water bath at 20 C. At zero time, 0.1 ml 0.0181 M ATP-Na_2 (P-L Biochemicals Inc., Milwaukee, Wisc.) was added to the reaction mixture. The reaction was stopped after 2, 4, 6, 8, and 10 min, respectively, by adding 2.0 ml 20% trichloroacetic acid (TCA) solution. The blank contained the same reagents as the reaction mixture, however, the TCA was added before the ATP solution. The protein precipitate was removed by centrifugation. Inorganic phosphate in 1.0 ml of the supernate was determined by the method of Rockstein and Herron (1951).

ATPase Activity as a Function of pH

The above assay procedure was used employing the following buffer solution: 0.31 M KCl – 0.09 M citrate at pH 5.0 and 5.5; 0.45 M KCl – 0.05 M histidine at pH 6.5 and 6.8; 0.45 M KCl – 0.05 M tris (hydroxymethyl) aminomethane at pH 7.0, 7.5, 8.0, 8.5, and 9.0, and 0.45 M KCl – 0.05 M glycine at pH 10 and 10.5.

The order of the ATPase inactivation with respect to protein concentration was carried out by incubating reaction mixtures containing from 0.25 to 1.0 mg of protein at 20.5 C for 20 min. Determination of the ATPase activity was then carried out as described above. The order of a reaction with respect to any given reactant is the exponent (n) to which the concentration of that reactant enters in the rate expression Rate = kC^n. The order (n) in these experiments is obtained from the slope of the graph where log v is plotted against log (P), v being the initial velocity and (P) the initial enzyme concentration.

Acetylcholinesterase Activity

The method of Hestrin (1949) was used to determine the acetylcholinesterase activity of myosin. The incubation mixture contained 2.0 mg protein

and 0.5 ml 4×10^{-3} M acetylcholine. The reaction was allowed to proceed for 10 min at 20 C.

Conductivity Measurements

Determination of the ionic strength was carried out with a conductivity meter (Conductivity Bridge, Model RC 16 B2, Industrial Instruments Inc.) which had previously been calibrated at 0 C against KCl solutions of known ionic strength.

RESULTS

Ultracentrifugation

At infinite dilution, the corrected sedimentation coefficient of trout myosin, $S^\circ_{20,w} = 6.50$, was in good agreement with the values reported for cod and rabbit myosin ($S^\circ_{20,w} = 6.43$) by Connell (1958) and by Johnson and Rowe (1960). At 0.25% protein concentration, the corrected sedimentation coefficient, $S_{20,w} = 5.75$, seemed to be identical with the value obtained for cod myosin. Sedimentation patterns at this protein concentration, at 5 C, are shown in Fig. 1. The tendency of trout myosin to aggregate readily is demonstrated in the sedimentation patterns obtained at 20 C (Fig. 2). Faster-moving, small peaks appeared in addition to the myosin component at this higher temperature. These aggregates sedimented to the bottom of the cell between 16 and 32 min.

Spectral Properties

The optical density ratio for DMSO-treated trout myosin was $E_{278}/E_{260} = 1.37$. Trout myosin which had not been treated with DMSO had E_{278}/E_{260} ratios around 1.16 (Fig. 3). This indicates that some purification from nucleic acid–nucleotide fractions was achieved. However, a second treatment with

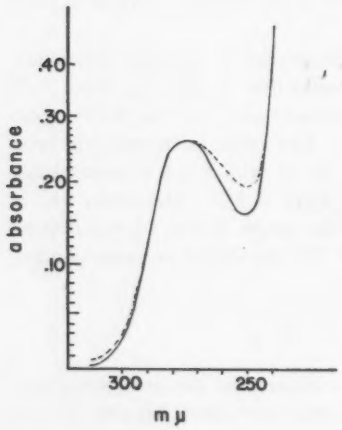

FIG. 3. Ultraviolet spectrum of trout myosin. *Solid line:* DMSO-treated trout myosin ($E_{278}/E_{260} = 1.37$); *broken line:* trout myosin prepared without solvent treatment ($E_{278}/E_{260} = 1.16$).

DMSO did not increase the optical density ratio any further. Only 0.06% of acid-soluble nucleotides were found to be associated with the DMSO-treated protein.

Enzyme Activity

The ATPase activity of myosin preparations treated with 15% DMSO varied between 0.40 to 0.60 μmoles P_i/mg prot. N/min (μ = 0.5, *p*H 6.8, 20 C). The acetylcholinesterase activity of the same preparations was between 0.35 to 0.45 μmoles acetylcholine/mg prot. N/min (*p*H 6.8, 20 C). The enzyme assays were carried out under zero order reaction conditions with respect to substrate and resulted in a linear relation for the rate of hydrolysis. Adenylic acid (AMP) was not deaminated by the myosin preparation.

The effect of DMSO on the ATPase activity of trout myosin was not investigated. The ATPase activity for ultracentrifuge cod myosin (prepared from actomyosin by addition of ATP–$MgCl_2$) was reported to be between 1.8 and 3.0 μmoles P_i/mg prot. N/min at an ionic strength μ = 0.10, *p*H 7.3 (Dingle and Hines, 1960). This is about 4 to 5 times greater than the activity of trout myosin reported here. However, the difference in ionic strength alone does not make this a very good comparison.

*p*H-Activity Curve

The ATPase activity of trout myosin has two optima, one around *p*H 6.5 and the other at about *p*H 10.0 (Fig. 4). The optimum at *p*H 10.0 has a larger apparent activity.

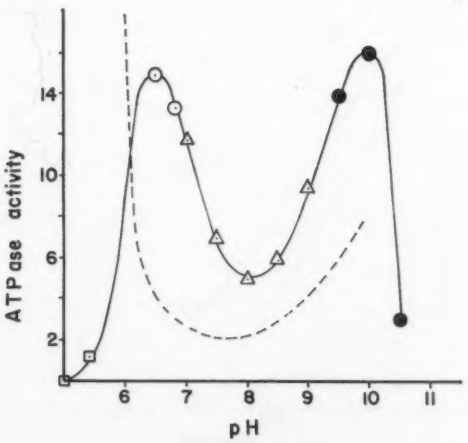

FIG. 4. *Solid line:* *p*H-activity (ATPase) curve of trout myosin; *broken line:* the rate of aggregation of cod myosin (Connell, 1960).

Stability

The ATPase activity of DMSO-treated trout myosin did not decrease during 22 days of storage at −1 C (μ = 0.5, KCl–histidine, *p*H 6.8, protein concentration 0.60%). The ATPase activity of trout myosin prepared without DMSO treatment began to decrease slowly after 14 days of storage at −1 C.

Heat Denaturation

Using the decrease in enzymatic activity as a measure of the changing protein structure, heat denaturation of trout myosin was studied between 12 and 25 C. The logarithm of the per cent remaining enzyme activity plotted against the time interval during which trout myosin had been exposed to a certain temperature shows a linear relation. The straight line indicates that the loss of enzymatic activity is a first-order reaction with respect to time (Fig. 5). At lower protein concentrations, the enzyme activity was lost at a greater rate. Oxygen or nitrogen equilibrated with the protein solutions during the incubation at 25 C did not change the rate at which the enzyme activity was lost.

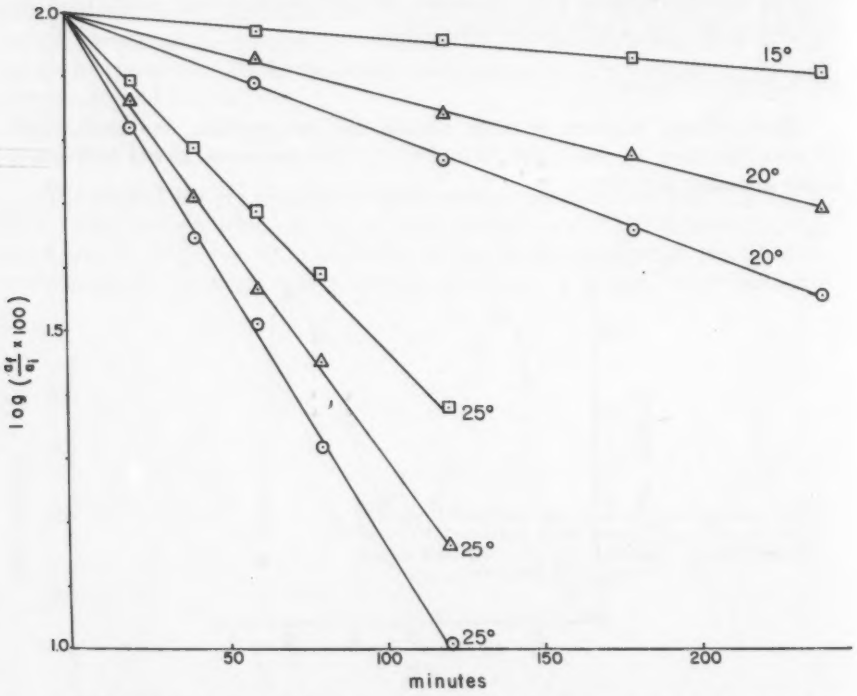

FIG. 5. Thermal inactivation of trout myosin ATPase at 15, 20, and 25 C and at protein concentrations of 0.25% (— ○ —), 0.52% (— △ —), and 0.625% (— □ —); ionic strength μ = 0.50, *p*H 6.8. The logarithm of the per cent remaining activity is plotted as a function of time.

The rates of inactivation of the myosin ATPase and the myosin acetylcholinesterase were different. Keeping the myosin solution at 25 C for 6 hr did not decrease the acetylcholinesterase activity. After increasing the incubation temperature to 45 C, the rate of acetylcholinesterase inactivation ($k = 5.77 \times 10^{-4}$ sec^{-1}) became comparable to the rate of inactivation of the ATPase activity ($k = 3.25 \times 10^{-4}$ sec^{-1}) at 25 C.

The first-order rate constants k for the enzyme inactivation were calculated from the half life of the inactivation reactions (Fig. 5). The Arrhenius plot shows a linear relation between the logarithms of the rate constants and the reciprocal of the absolute temperature (Fig. 6). The slopes for different protein concentrations, obtained from different myosin preparations, were approximately the same. The activation energy (E) was 46 kcal mole^{-1} at pH 6.8. The enthalpy of activation (ΔH^*) was then 45.4 kcal mole^{-1} and the entropy of activation ΔS^*, 77 e.u. (entropy units in cal/degree/mole). The

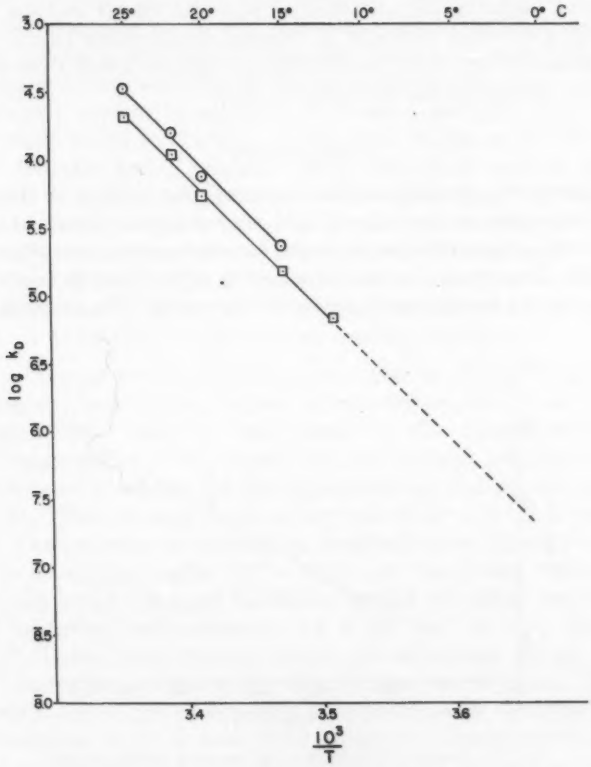

FIG. 6. Effect of temperature on the rate of inactivation of trout myosin ATPase. Protein concentrations 0.25% (— ○ —) and 0.625% (— □ —).

temperature coefficient, $Q_{10} = \frac{k_t + 10^\circ}{k_t}$, for the myosin ATPase inactivation was found to be a relatively high value. Between 15 and 25 C, the Q_{10} for trout myosin was about 15 (protein concentration 0.625%). From the data of Pelletier and Ouellet (1961), we calculated a Q_{10} ratio of 25 for the inactivation of myosin ATPase in the same temperature range but at a lower protein concentration (0.06%). Similar calculations could not be carried out for cod myosin ATPase, since the kinetics of inactivation have been studied at 0 C only.

Extrapolating the straight lines in the Arrhenius plot back to 0 C, we obtained values for k around 1.90×10^{-7} sec^{-1} for trout myosin (Fig. 6). Using the same procedure on the data of Pelletier and Ouellet (1961), k was about 4.75×10^{-9} sec^{-1} for rabbit myosin. Plotting the data of Connell (1960), at 0.2% protein concentration, gives the first order rate constant 4.31×10^{-6} sec^{-1} for the inactivation of cod myosin ATPase at 0 C. The properties of trout myosin are in many respects similar to cod and rabbit myosin. Differences in the stability of these myosins to thermal inactivation of the enzyme activity are, however, apparent. Rabbit myosin is more stable than trout myosin which in turn is more stable than cod myosin.

DISCUSSION

ATPase activity of rabbit myosin has been associated with H-meromyosin, and acetylcholinesterase activity with L-meromyosin (Kövér et al., 1964a). The isolation of a cholinesterase from fish muscle has also been reported (Kövér et al., 1964b). The results in this paper seem to indicate that acetylcholinesterase is part of the myosin molecule from fish muscle. The acetylcholinesterase

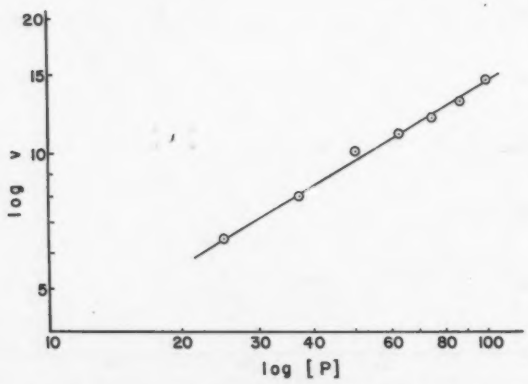

FIG. 7. Graph to determine the reaction order of the ATPase inactivation with respect to protein concentration. The logarithm of the inactivation rate (μg P_i produced) is plotted against the logarithm of the relative protein concentrations.

activity of trout muscle was also shown to be more sensitive to thermal denaturation than that from rabbit myosin. Acetylcholinesterase activity associated with trout myosin is lost fairly rapidly at 45 C ($t_{\frac{1}{2}}$ = 20 min) while rabbit myosin can be heated for short time periods at 48 C without a notable loss of acetylcholinesterase activity.

The *p*H-activity curve of trout myosin is similar to that of rabbit myosin. The minimum activity, between *p*H 7.5–8.5, seems to correspond to the minimum aggregation reported for cod myosin (Connell, 1960). However, at *p*H 6.8, the point at which the ATPase activity was measured, aggregation is still at a low level. The significance of the alkaline *p*H optimum of rabbit myosin has been discussed (Kielly, 1961).

Heat denaturation as expressed by the loss of enzymatic activity (ATPase) is decreased at higher protein concentrations. This effect of protein concentration on denaturation has been explained as "protection" and is a general phenomenon for most enzymes and proteins. Another explanation is that the predominant species of protein molecules present at high concentrations are less susceptible to thermal inactivation. Such a concentration effect can also explain the higher stability of fish proteins in the muscle as compared to the extracted protein in solution. Activation energies greater than 210 kcal mole^{-1} were found in thermal inactivation studies of ATPases in whole fish muscle (Nemitz and Partmann, 1959). For crude enzyme preparations the energy of activation is generally much higher than for those that are highly purified. However, the reason that the activation energy of trout myosin (46 kcal mole^{-1}) is lower than that of rabbit myosin (54 kcal mole^{-1}) is probably due to its lower stability. The differences between the rate constants for the ATPase inactivation of trout, rabbit, and cod myosin, at a particular temperature, give an indication of the relative protein stabilities.

The structure of myosin in solution can also be altered by intermolecular reactions whereby myosin polymerizes to form dimers and trimers, etc. (Lowey and Holtzer, 1959). Studying cod myosin in the ultracentrifuge, Connell found that aggregation is accelerated at high protein concentrations. Using the first-order rate constants for the aggregation of cod myosin at 0, 3.5, and 8 C (Connell, 1960), an activation energy of about 21.6 kcal mole^{-1} can be calculated. The entropy of activation, obtained from Eyring's relation, was found to be a negative value, $\Delta S^* = -8.6$ e.u. Using rate constants for the increase of the weight average molecular weight of rabbit myosin, obtained from light scattering measurements, 1.8×10^{-5} sec^{-1} at 25 C and 1.8×10^{-6} sec^{-1} at 4 C (Lowey and Holtzer, 1959), an activation energy of about 21 kcal mole^{-1} and a negative entropy of activation are obtained. The negative entropy values are in agreement with a polymerization reaction in which the activated complex forms a more rigid structure. Denaturation measured by the loss of myosin ATPase activity proceeds with positive entropy changes and can be looked upon as an opening or unfolding of the secondary and tertiary structure of the myosin molecule.

The polymerization reactions described by Lowey and Holtzer (1959) and by Johnson and Rowe (1961) are irreversible. However, it has recently been demonstrated that cod myosin can also undergo a reversible endothermic type of aggregation at $\mu = 0.10$ (Mackie and Connell, 1964). This type of aggregation may turn out to be similar, for instance, to the polymerization reaction described for the coat protein from tobacco mosaic virus. At room temperature, *p*H 6.5, and $\mu = 0.1$, this protein associates reversibly with a large increase in turbidity and the release of water. Dissociation is brought about in the cold and occurs with clarification of the solution. It is reasoned that the polymerization reaction involves the release of water from the binding sites of the hydrated protein surfaces. The difference in entropy between the dehydration and the immobilization of monomer units into dimer and higher polymer structures turns out to be a large positive value (Ansevin et al., 1964).

The stability of trout myosin to denaturation seemed greater at −1 C than was expected from studies at higher temperatures (12–25 C). Denaturation of myosin at temperatures below 0 C therefore seems to follow a different mechanism and deserves further attention.

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Distribution and Speciation of Iron in Natural Waters: Transition from River Water to a Marine Environment, British Columbia, Canada[1,2]

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ABSTRACT

Total and "HCl reactive" iron has been determined for particulate and soluble forms in freshwater and seawater samples along a profile extending from Hope, British Columbia, 100 miles up the Fraser River, to a station in the Northeast Pacific Ocean, 60 miles west of Cape Flattery, Washington. Complementary data were taken on salinity, dissolved oxygen, *p*H, and temperature. Observations covered periods of high and low river runoff from June 1962 to June 1963.

Total particulate iron concentrations in the Fraser River were highest during the maximum runoff period in summer, while total soluble iron concentrations did not significantly increase at this time. Admixture of saline water with fresh water at the river mouth caused the expected abrupt decrease in particulate iron below the upper 2 m.

Both the total particulate and total soluble iron concentrations in seawater samples decreased from the Strait of Georgia seaward through Juan de Fuca Strait out into the Pacific Ocean. In summer, the total soluble iron was higher than in winter while the total particulate iron was lower. The seasonal fluctuations of the sum of the total particulate and total soluble iron were not significant.

The high amounts of the soluble forms of iron and the concurrent low particulate iron concentrations in summer may be partially explained by the assimilation of particulate iron during periods of high phytoplankton productivity, mainly by diatoms, with release of soluble forms during lysis of these organisms.

The mean annual total iron discharged by the Fraser River was 1.90×10^6 kg/day, the bulk of which was immediately laid down in the river delta.

There was no evident correlation of the iron distribution with that of *p*H, oxygen, or density, excepting in the region immediately adjacent to the mouth of the Fraser River.

INTRODUCTION

In an approach to the problem of organically complexed iron in sea water, a form postulated to occur in natural waters but never identified (Cooper, 1937; Shapiro, 1957, 1964; Hutchinson, 1957), a series of iron determinations was made on freshwater and seawater samples in a river — coastal marine — open oceanic environment. The results presented here, however, are of more significance in evaluating the seasonal influx of soluble and particulate forms of iron into the marine environment from the Fraser River and the super-

[1]Received for publication August 16, 1965.
[2]This work was supported by the National Research Council of Canada under Block Term Grant BT-100, and by two Summer Research Grants from the University of British Columbia, 1962 and 1963.
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J. Fish. Res. Bd. Canada, 23(4), 1966.
Printed in Canada.

imposed effects of phytoplankton production upon the iron distribution, than in delineating organic-iron complexes.

Published analyses of soluble and particulate iron in marine waters have been summarized by Lewis and Goldberg (1954) up to 1953. More recent work includes: total iron near Plymouth, England, in the English Channel, and in the Bay of Biscay (Armstrong, 1957); particulate and soluble iron in Puget Sound, Washington, and the Juan de Fuca Strait (Laevastu and Thompson, 1958); particulate and soluble iron in the Sargasso Sea (Menzel and Spaeth, 1962); total iron in Chesapeake Bay (Sanannman and Lear, 1961); particulate iron in the Gulf of Panama (Schaefer and Bishop, 1958); particulate and soluble iron in the Florida Current and Biscayne Bay (Corcoran and Alexander, 1964); and particulate iron in the coastal waters of the Pacific Northwest (Joyner, 1964). There exists little published data on the soluble forms of iron in rivers. Strakhova (1948) reports variable and high ratios of particulate to soluble iron in Russian rivers and inland seas. Total particulate iron concentrations in rivers and other fresh water have been reviewed by Livingston (1963), Woodward and Heidel (1964), and Durum et al. (1960). Particulate iron concentrations in the Fraser River have been reported by Durum et al. (1960) and Leverin (1947). Thompson and Bremner (1935) analyzed for total and soluble iron in the Juan de Fuca Strait, the Strait of Georgia, and in the Puget Sound area in the Northeast Pacific Ocean. Laevastu and Thompson (1958) added to this data for the inland waters of Washington and determined the ratio of iron to total inorganic particulate matter. Thompson and Bremner (1935) demonstrated high total and soluble iron concentrations in the winter months, during periods of low river runoff and decreased phytoplankton production. Joyner (1964) showed that the Columbia River plume may be traced northward by the decreasing concentrations of particulate iron in surface water, and also reported particulate iron values for samples taken in vertical profiles at several stations off Vancouver Island.

METHODS

The four forms of iron determined in this work give an arbitrary breakdown of particulate, colloidal, and dissolved species. The initial filtration through a 0.45-μ filter, giving the particulate fraction, included iron in and associated with terrigenous inorganic minerals, large aggregates of hydrated iron oxides, and iron in and associated with dead and living phyto- and zooplanktonic organisms and their detrital by-products. Treatment of this particulate material with 0.5 N HCl at 100 C for 10 min will liberate iron from the hydrated iron oxides, and to an unknown degree, iron from the terrigenous components and from the living and dead organic contributions. The more refractory clay minerals and highly stable organic complexes of iron will not be attacked. The filtrate includes: soluble Fe^{3+}, $Fe(OH)^{2+}$ at concentrations less than 0.5 μg/litre (Cooper, 1948), colloidal and soluble hydrated iron oxides, and colloidal and soluble organic iron complexes. The HCl-reactive

soluble-iron is a measure of iron likely to be available to marine phytoplankton and may include soluble organic iron complexes as well as colloidal forms of ferric hydroxide.

Sampling Locations

Figures 1 and 2 show stations in the oceanic and river environment, respectively. Table I gives cruise numbers, locations, and bottom depths. No attempt was made to sample at times corresponding to any particular tidal period.

Collection of Samples

Seawater was collected in either 3-litre polyvinylchloride van Dorn bottles or in 1-litre polypropylene N10 bottles (available from the National Institute of Oceanography, Wormley, Godalming, England), using a stainless steel hydrographic wire. All metal fastenings, messengers, etc., were sprayed with acrylate polymer. The samplers were initially cleaned with 6 N HCl, and with 1 N HCl between stations. Duplicates are samples taken from each of two lowerings of the same bottle to the same depth. Every feasible precaution was

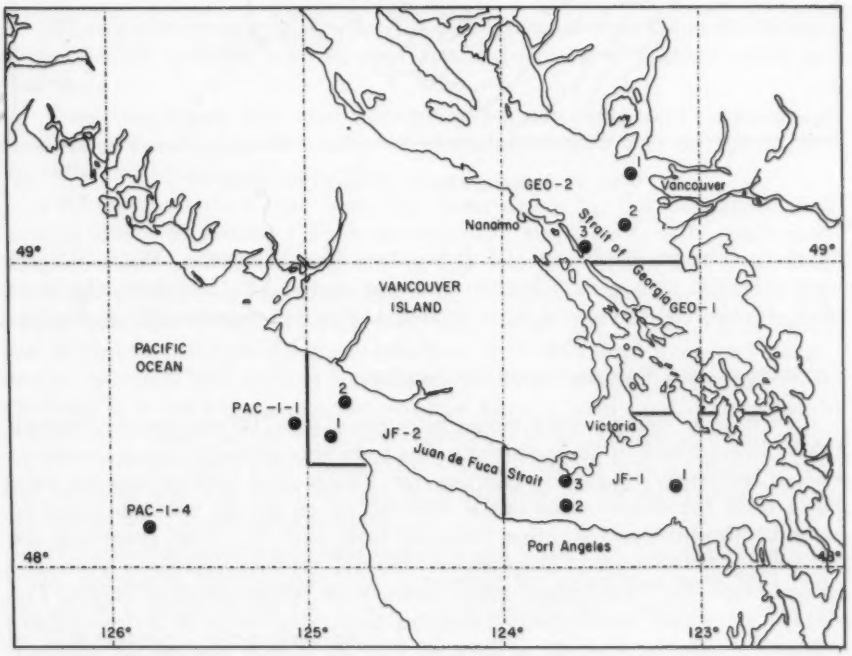

FIG. 1. Sampling locations in the Strait of Georgia, Juan de Fuca Strait, and the Pacific Ocean.

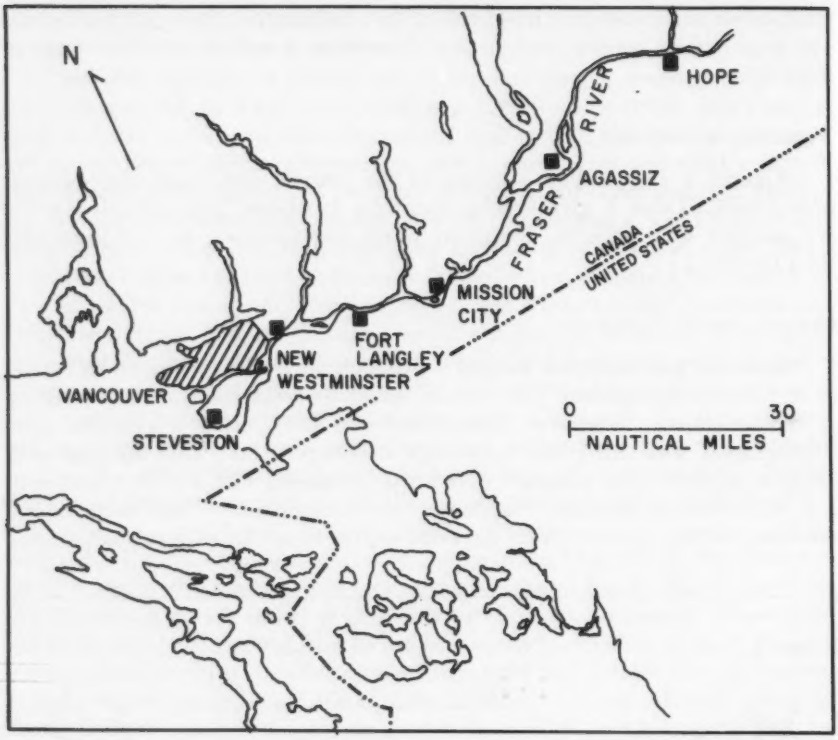

FIG. 2. Sampling locations in the Fraser River.

taken to prevent shipboard and atmospheric contamination. River samples were collected in a plastic bucket from the surface, 8–15 ft from the river bank, transferred to polyethylene bottles, and frozen immediately in dry ice.

FILTRATION AND PRESERVATION OF SAMPLES

Particulate iron was collected by filtration of 500–1000-ml samples through HA millipore filters (0.45-μ pore size). The pads were placed in paper envelopes and subsequently analyzed. Samples for soluble iron determinations were taken from the filtrate. The initial 400–500 ml of filtrate were discarded in order to prevent contamination from the filter pad. A 125-ml sample of the filtrate was transferred to polyethylene bottles, previously cleaned with hot, concentrated HCl and rinsed three times with double-distilled water. The samples were then immediately frozen in either dry ice or in a deep freeze. Internal standards, 4 or 80 μg/litre of iron as ferrous ammonium sulphate were added to random samples to assess percentage recoveries of soluble iron.

TABLE I. Station locations; Cruises 62/16 (June 21-22, 1962), 62/19 (July 12-14, 1962), 63/7 (Feb. 26 to Mar. 5, 1963), 63/20 (June 5-7, 1963).

Station	Location	Bottom depth (*m*)
Geo-2-1	49°17.0′N, 123°18.6′W	113
Geo-2-2	49°01′N, 132°20.5′W	225
Geo-2-3	49°02.7′N, 123°33.6′W	198
JF-1-1	48°15′N, 123°20.3′W	132
JF-1-3	48°17′N, 123°40′W	161
JF-1-2	48°12.5′N, 123°40′W	154
JF-2-1	48°26′N, 124°51′W	247
JF-2-2	48°33.6′N, 124°51′W	95
Pac-1-4	48°08′N, 125°50′W	1100

ANALYTICAL PROCEDURES

All reagents were freed of iron by distillation, extraction as the bathophenanthroline complex with isoamyl alcohol, or recrystallization when applicable.

Total particulate iron was determined by perchloric acid oxidation of the filter pads following the method of Lewis and Goldberg (1954) using 2,2′-bipyridine as the complexing agent.

HCl-reactive particulate iron was determined by the Strickland and Parsons (1960) procedure. Iron is leached from the filter pads by treatment with 0.5 N HCl at 100 C for 10 min and is determined with 2,2′-bipyridine.

Total soluble iron was determined by autoclaving the filtered seawater sample (acidified to *p*H 0.15 with HCl) for 5 hr at 125 C. The Vycor flasks used in this determination were conditioned with HCl until the blank values became constant. This method is similar to that reported by Armstrong (1957) excepting that the autoclaving temperature was 15° lower and bathophenanthroline was used instead of 1,10-phenanthroline as the complexing agent and the colored complex was concentrated by extraction into isoamyl alcohol. This method was also used for the determination of the total soluble plus particulate iron in unfiltered samples.

HCl-reactive soluble iron was determined by the Strickland and Parsons (1960) technique. The soluble iron is released by treatment with HCl, *p*H 1.5, at room temperature for 5 min and extracted into isoamyl alcohol as the bathophenanthroline complex.

Turbidity corrections were applied to all results when applicable.

Controls and Standards

Soluble and particulate iron standards were run each day. Recovery of iron standards added to the frozen samples is shown in Table II. Replicate samples, sample bottle controls, and filter pad blanks are listed in Table III. The high total particulate iron values found in one set of duplicates for the double-distilled water leachate of a van Dorn bottle was taken to be spurious contamination.

Results and Discussion

Tables IV–IX give the total and HCl-reactive iron concentrations in the soluble and particulate fractions for all samples taken. Duplicate values are included to demonstrate the variation between samples taken at the same depth. The total particulate iron values for the river samples (Table IV) show a wider spread than the seawater samples (Table VIII). This is due to

Table II. Recovery of iron standard added to filtered seawater aliquots prior to freezing.

	μg Fe/liter recovered	
μg Fe/liter added	Soluble (total)	Soluble (HCl-reactive)
80	78.8	76.5
80	76.9	71.1
80	71.5	77.2
80	79.8	70.0
80	79.6	59.8
80		62.0
Mean:	77.5	69.4
Percentage recovery	97	87
4	3.3	2.4
4	2.7	2.6
4	3.4	3.1
4	2.4	3.6
Mean:	3.0	2.9
Percentage recovery	75	72

TABLE III. Replicate analyses, controls, and blanks (all values in μg Fe/liter).

Replicate analyses

	Total (autoclaved)[a]		HCl-reactive[b]		Total[c]
	(Soluble)	(Sol. + Part)	(Soluble)	(Particulate)	(Particulate)
	2.7	5.1	3.1	3.5	17.5
	3.5	7.1	3.2	2.5	19.0
	3.7	8.2	3.5	3.2	18.7
	3.3	6.0	3.5	3.1	
	2.8	6.6	2.8	3.1	
			2.5		
Mean:	3.2	6.6	3.1	3.1	18.4
SD	±0.4	±1.0	±0.4	±0.4	±0.8

Sampling bottle controls[d]

	Van Dorn	NIO
HCl-reactive soluble	0.0, 0.2	0.0, 0.0
Total soluble	0.0, 0.3, 0.3	0.0, 0.0, 0.4, 0.5
HCl-reactive particulate	0.7, 0.6	0.0, 0.0
Total particulate	2.6, 2.4, 0.3, 0.6	0.0, 0.0

Filter pads[e]

HCl-reactive particulate	0.4±0.1
Total particulate	0.8±0.2

[a]Aged sea water from cruise 63/20, station JF-1-1, 20 m.
[b]Filtered and aged aquarium sea water.
[c]Samples from 20 m, cruise 63/20, station JF-1-1.
[d]Doubled-distilled water allowed to stand 15 min in sampling bottle and carried through the analysis. Values corrected for traces of iron in the distilled water and filter pads.
[e]Results run on duplicate pads from each filter box. Variations shown are between boxes.

a partial loss of particles from the dried filter pads into the storage envelope, particles being included in one determination and not the other. At station JF-1-1, replicate samples were taken by six successive lowerings of the NIO bottle to 20 m (Table X). The total soluble values show the widest scatter of the four forms determined, and for samples taken on cruise 62/16 were generally lower than the HCl-reactive iron concentrations. This discrepancy was likewise observed for 14 scattered samples from cruises 62/19, 63/7, and 63/20. This problem was not resolved and thus limits interpretations of total soluble iron results notably for cruise 62/19.

A comparison of total particulate iron plus total soluble iron with the total iron in unfiltered samples is shown in Table XI. These results, with the exception of one 50-m set, are not inconsistent with variations found in all autoclaved samples, and may represent a more contagious distribution of the total soluble-iron rather than experimental error (see Table III).

The mean values for each of the four forms of iron in sea water are depicted in Fig. 3 and 4 and illustrate the distribution in late February to early

TABLE IV. Fraser River samples (all values μg Fe/liter).

		Soluble		Particulate	
		HCl-reactive	Total	HCl-reactive	Total
Hope	6/25/62	4.9, 4.9	10.8, 11.5	363, 372	–
	8/9/62	2.8, 0.0	6.6, 7.4	253, 254	710
	12/28/62	–	–	–	–
	6/8/63	7.9, 5.4	14.3, 12.4	216, 244	622, 625
Agassiz	6/25/62	9.9, 8.4	11.8, 13.4	1200, 1280	–
	8/9/62	8.1, 0.0	12.4, 11.9	1360, 1330	1835
	12/28/62	8.2, 8.0	17.5, 18.0	322, 314	825, 100
	6/8/63	4.4, 3.7	14.2, 73.1	692, 702	1070, 746
Mission	6/25/62	4.9, 6.3	41.7, 14.3	1140, 1100	–
	8/9/62	7.2, 10.3	11.5, 9.5	1210, 1070	2290
	12/28/62	9.3, 8.8	16.3, 16.0	330, 296	410, 450
	6/8/63	3.6, 4.0	28.6, 12.1	728, 696	770, 1060
Fort Langley	6/25/62	4.7, 7.1	10.5, 18.3	1120, 1330	–
	8/9/62	6.5, 0.0	18.3, 10.7	1170, 1320	2450
	12/28/62	7.2, 7.1	18.0, 34.0	333, 301	373, 710
	6/8/63	3.0, 3.9,	7.6, 10.6	664, 696	790, 1000
New Westminster	6/25/62	5.4, 11.6	11.5, 12.6	754, 874	–
	8/9/62	19.4, 11.6	9.6, 9.4	1260, 990	2118
	12/28/62	10.3, 10.0	25.4, 22.1	325, 373	410, 580
	6/8/63	4.8, 2.9	29.8, 27.5	748, 752	760, 1147
Steveston	6/25/62	14.7, 14.0	36.9, 40.7	849, 868	–
	8/9/62	–	–	–	–
	12/28/62	1.2, 21.2	25.5, 25.5	462, 435	470, 466
	6/8/63	7.9, 5.4	19.4, 19.1	620, 542	830, 960

March 1962, and in July 1963. Both the total and HCl-reactive soluble iron are higher in July than in March while the total and HCl particulate iron is lower during the summer. These low particulate iron values persist although this represents the time of maximum river runoff with its associated high input of particulate iron (Table XII). Scavenging of particulate iron by diatoms (Goldberg, 1952), the dominant phytoplankters present in summer blooms in this area, may effectively keep the concentration of particulate iron low. Lysis of the diatoms then releases soluble-iron into the water.

Figures 3 and 4 illustrate the gradation from high to low values for both the HCl-reactive and total particulate iron from the mouth of the Fraser River 60 miles out into the Pacific Ocean. The HCl-soluble iron shows remarkably little gradation along this traverse.

In Table XIII, ratios of total particulate iron to HCl-reactive particulate iron and of total soluble iron to HCl-reactive soluble iron are given for the entire water column at each individual station. These ratios vary by a factor of five but yield mean seawater ratios equal to 2.5 for the particulate iron and 2.3 for the soluble iron. This indicates that treatment with dilute HCl

TABLE V. HCl-reactive soluble iron (μg Fe/liter).

	Stations							
	Geo-2-1	Geo-2-2	Geo-2-3	JF-1-1	JF-1-3	JF-1-2	JF-2-1	JF-2-2
Cruise 62/16								
Surface	4.0, 7.6	13.4, 8.1	8.2, 5.4					
5 m	5.4, 2.4	5.8, 6.8	5.7, 7.2					
10 m	0.0, 1.2	10.6, 10.6	11.8, 6.1					
20 m	8.4, 3.3	19.6, 10.5	10.0, 7.0					
50 m	1.0, 1.0	6.0, 8.8	6.7, 6.8					
75 m	1.9, 2.9	5.3, 13.9	7.2, 3.3					
125 m			12.1, 6.1					
Cruise 62/19								
Surface		3.9, 1.9	4.3, 19.8	4.5, 9.8	7.6, 5.8	6.3, 3.3	6.8, 10.0	4.8, 4.7
5 m		4.8, 3.7		5.3, 4.2				
10 m				2.4, 6.8,				
20 m		8.3, 7.8		2.4, 2.9	2.3, 5.8	4.9, 5.4	7.2, 8.9	11.3, 11.9
50 m		5.7, 4.7		5.3, 3.5	9.0, 6.3	2.2, 4.1	5.9, 4.6	10.3, 10.9
75 m		6.7, 3.8		5.2, 4.8	4.1, 2.3	5.1, 5.3	6.0, 7.2	
125 m		7.6, 6.5			3.3, 5.2	5.3, 6.2	9.2, 6.2	5.1, 6.7[a]
Cruise 63/7								
Surface		1.6, 2.6		2.9, 1.9	1.7, 0.8	1.2, 0.6	0.9, 1.2	2.6, 0.0
5 m		1.6, 1.2		1.9, 1.7				
20 m		1.9, 1.4		1.9, 1.5	0.9, 0.8	1.4, 0.6	1.4, 1.7	1.5, 1.2
50 m		0.9, 1.9		2.2, 3.3	0.7, 0.4	0.5, 0.6	1.4, 0.0	1.9, 0.8
75 m				0.9, 1.9	1.2, 0.7	0.0, 0.0	2.0, 0.3	1.2, 1.0
100 m				2.0, 0.7			0.1, 0.7	
125 m		1.6, 1.3			0.3, 0.1	1.0, 0.0	1.3, 0.3	
200 m							1.2, 0.9	
Cruise 63/20								
Surface		7.8, 3.0			3.5, 1.8			
5 m		2.3, 2.4		2.2, 3.1	3.3, 3.1			
20 m		2.4, 2.9, 3.0, 3.3		2.4[b]	0.3, 2.4, 4.8, 4.4			
50 m		2.5, 2.3			2.6, 0.4			
75 m		2.6, 1.3, 2.3, 2.0		3.1, 2.3,	3.0, 3.7			
125 m		3.0, 3.0		2.6, 3.2	2.0, 1.9, 1.9, 2.4			

[a]One hundred meters.
[b]Mean of six bottle lowerings (see Table X).

TABLE VI. Total soluble iron (μg Fe/liter).

	Stations							
	Geo-2-1	Geo-2-2	Geo-2-3	JF-1-1	JF-1-3	JF-1-2	JF-2-1	JF-2-2
Cruise 62/16								
Surface	5.1, 5.1	6.7, 10.6	4.1, 12.4					
5 m	35.5, 20.4	7.0, 9.3	4.8, 7.3					
10 m	6.2, 10.9	9.9, 1.3	3.9, 5.1					
20 m	25.1, 6.1	1.8, 3.2	7.1, 27.0					
50 m	4.5, 5.6	2.0, 2.7	3.8, 3.0					
75 m	6.2, 6.8	2.6, 3.8	3.4, 3.5					
125 m			5.1, 5.5					
Cruise 62/19								
Surface		6.1, 9.3	8.7, 5.6	12.2, 15.1	6.3, 17.0	16.6, 33.6	12.0, 7.4	1.7, 0.8
5 m		5.5, 5.6		6.9, 9.0				
10 m				4.7, 9.0				
20 m		7.8, 9.6		12.1, 6.9	4.8, 4.2	10.7, 21.5	10.5, 14.5	1.0, 5.6
50 m		7.2, 5.1		7.1, 9.9	38.6, 16.2	11.9, 10.6	16.8, 12.1	5.4, 5.1
75 m		8.6, 8.7		6.8, 10.9	19.8, 18.0	5.8, 18.5	4.1, 3.8	
125 m		7.9, 7.1			27.1, 24.3	16.9, 15.9	10.4, 12.3	9.4, 7.6[a]
Cruise 63/7								
Surface		7.2, 8.3		5.3, 1.9	5.8, 5.7	2.1, 2.1	0.6, 2.6	15.3, 3.6
5 m		4.8, 4.7		1.0, 3.0				
20 m		0.7, 2.4		2.2, 1.4	9.1, 7.2	1.8, 3.4	4.4, 2.8	0.3, 1.6
50 m		0.0, 0.7		0.5, 1.1	1.7, 1.2	3.8, 2.4	1.3, 0.7	1.4, 0.8
75 m				1.6, 2.5	4.7, 4.5	1.4, 1.6	2.1, 2.2	1.2, 1.2
100 m				1.8, 1.7			1.2, 6.0	
125 m		2.2, 1.3			2.7, 2.5	1.6, 1.6	1.1, 1.2	
200 m							1.1, 1.4	
Cruise 63/20								
Surface		11.9, 11.3			7.2, 6.6, 6.9, 4.3, 8.8			
5 m		18.3, 24.1		4.0, 5.7, 7.2	18.7, 11.1			
20 m		5.5, 2.0		11.4[b]	5.8, 4.8			
50 m		18.0, 8.4			4.8, 21.5			
75 m		7.8, 4.4		33.5, 2.9	6.5, 3.9			
125 m		3.0, 12.3		14.6, 14.9, 3.5, 5.0	11.9, 9.4, 4.8			

[a]One hundred meters.
[b]Mean of five bottle lowerings (see Table X).

TABLE VII. HCl-reactive particulate iron (μg Fe/liter).

	Stations							
	Geo-2-1	Geo-2-2	Geo-2-3	JF-1-1	JF-1-3	JF-1-2	JF-2-1	JF-2-2
Cruise 62/16								
Surface	193, 199	264, 224	75.0, 76.5					
5 m	42.2, 56.0	236, 296	31.0, 31.8					
10 m	29.3, 30.8	97.0, 141	14.4, 18.1					
20 m	19.5, 19.5	40.0, 19.4	9.5, 11.8					
50 m	22.2, 31.5	11.2, 5.5	2.6, 7.4					
75 m	19.7, 14.2	17.9, 39.0	4.2, 3.2					
125 m			10.2, 5.6					
Cruise 62/19								
Surface		259, 223	295, 260	8.6, 3.6	5.5, 5.2	5.2, 7.6	1.3, 1.4	1.8, 1.2
5 m		19.5, 15.8		8.8, 6.9				
10 m				9.2, 8.9				
20 m		2.8, 3.3		7.4, 9.8	6.1, 5.6	7.3, 7.6	1.2, 1.2	1.9, 1.6
50 m		2.6, 2.0		11.1, 8.9	7.6, 6.6	5.1, 5.6	2.5, 0.9	3.0, 3.0
75 m		1.4, 2.9		8.9, 10.0	6.1, 9.8	5.3, 3.5	1.0, 2.0	
125 m		8.4, 6.0			22.8, 21.6	9.6, 4.6	3.1, 2.6	4.2, 2.9[a]
Cruise 63/7								
Surface		76.5, 64.0		11.7, 12.5	10.0, 10.9	10.4, 8.6	7.5, 6.3	8.5, 9.3
5 m		17.2, 18.1		10.7, 13.5				
20 m		8.9, 7.8		12.9, 11.4	14.1, 12.9	13.2, 12.6	12.7, 11.1	12.2, 12.5
50 m		7.6, 8.7		16.5, 12.8	18.8, 16.8	9.6, 9.1	8.3, 8.6	10.0, 9.3
75 m				20.4, 19.1	16.1, 15.3	8.1, 8.2	11.2, 10.1	29.3, 28.2
100 m				29.0, 25.4			6.9	
125 m		5.1, 15.7			17.6, 20.0	10.0, 10.3	8.1, 7.5	
200 m							9.2, 9.3	
Cruise 63/20								
Surface		100, 80.8			2.7, 2.9			
5 m		50.7, 23.3		4.5, 4.7	2.1, 1.6			
20 m		1.9, 2.0		6.5[b]	2.7, 3.2			
50 m		2.4, 2.4			4.6, 4.3			
75 m		4.3, 4.1		6.5, 7.5	5.2, 5.6			
125 m		8.9, 7.8		14.7, 13.1	5.1, 6.0			

[a]One hundred meters.
[b]Mean of three bottle lowerings (see Table X).

TABLE VIII. Total particulate iron (μg Fe/liter).

	Stations							
	Geo-2-1	Geo-2-2	Geo-2-3	JF-1-1	JF-1-3	JF-1-2	JF-2-1	JF-2-2
Cruise 62/16								
Surface	311, 378		172, 218					
5 m	50.6, 64.4	465, 512	77.8, 86.0					
10 m	54.7, 45.6	295, 202	49.5, 48.9					
20 m	44.0, 47.8	99.0, 318	32.4, 30.4					
50 m	66.4, 45.1		8.8, 13.3					
75 m	22.7, 35.9		11.6, 8.8					
125 m			10.6, 9.4					
Cruise 62/19								
Surface		195, 167	470, 466	15.9, 18.3	13.9, 14.7	14.2, 13.9	8.7, 9.2	4.4, 6.0
5 m		45.9, 57.1		20.4, 20.9				
10 m				20.6, 22.4				
20 m		7.4, 7.3		20.3, 20.9	14.4, 14.5	13.3, 14.5	5.6, 5.9	4.0, 4.4
50 m		6.1, 5.9		20.3, 20.8	15.8, 15.6	8.9, 11.5	4.2, 3.9	4.9, 6.0
75 m		8.3, 6.1		20.6, 19.8	22.5, 14.8	13.3, 14.5	7.3, 6.8	
125 m		13.6, 14.9			43.2, 43.1	14.2, 13.9	11.8, 10.6	6.1, 7.4[a]
Cruise 63/7								
Surface		173, 160		25.7, 28.2	20.5, 20.7	21.6, 23.5	18.2, 16.9	17.7, 19.9
5 m		35.4, 37.0		27.1, 28.5				
20 m		20.5, 18.8		34.0, 31.2	27.9, 25.7	30.7, 26.8	24.9, 22.7	16.3, 16.0
50 m		20.5, 18.2		31.0, 31.5	51.7, 41.8	20.2, 21.0	26.8, 28.5	17.1, 15.8
75 m				31.5, 34.3	31.1, 31.0	17.1, 18.8	31.5, 26.5	34.8, 43.7
100 m				56.1, 55.0			17.1, 18.0	
125 m		33.7, 40.4			48.9, 45.0	22.7, 25.4	21.3, 22.1	
200 m							27.1, 24.6	
Cruise 63/20								
Surface		706, 566			4.8, 5.2			
5 m		168, 121		12.8, 12.7	4.2, 2.3			
20 m		6.8, 5.6		16.2[b]	6.4, 6.6			
50 m		5.0, 6.6			10.6, 14.5			
75 m		9.5, 9.6		19.3, 22.9	12.5, 11.8			
125 m		16.6, 18.8		47.2, 38.8	12.4, 10.6			

[a]One hundred meters.
[b]Mean of three bottle lowerings (see Table X).

TABLE IX. Soluble and particulate iron values (μg Fe/liter), Station Pac-1-1 and Friday Harbor, San Juan Islands, Washington.

	Soluble		Particulate		
	HCl-reactive	Total	HCl-reactive	Total	Total (Mean)
Cruise 62/19					
Surface	2.0, 2.4	7.9, 24.3	0.0, 1.5	1.5, 0.8	17.3
20 m	2.0, 2.4	38.3, 12.4	0.0, 0.0	0.4, 0.5	25.9
50 m	2.1, 3.8	6.7, 5.7	0.2, 0.2	0.9, 0.5	6.9
75 m	3.9, 7.8	2.2, 3.4	0.3, 0.4	0.3, 0.5	3.2
125 m	5.7, 5.1	23.1, 7.1	0.5, 0.1	0.6, 0.8	15.8
225 m	7.1, 9.2	6.9, 5.8	0.3, 0.3	0.7, 0.8	7.2
325 m	5.8, 2.9	7.0, 9.4	0.8, 0.7	1.3, 1.3	9.5
425 m	2.9, 3.3	2.5, 6.6	2.2, 1.8	3.9, 3.8	8.5
Cruise 63/7					
20 m	0.2, 0.2	2.4, 6.7	0.9, 1.0	1.9, 2.4	6.8
50 m	0.0, 0.4	5.1, 1.7	2.2, 2.3	7.2, 4.9	9.5
125 m	0.2, 0.0	2.7, 3.0	3.3, 3.2	11.2, 17.0	17.0
200 m	0.0, 0.0	2.4, 2.6	2.4, 1.1	7.2, 4.9	8.6
400 m	0.8, 0.7	2.4, 2.4	0.0, 0.0	1.9, 2.4	4.6
Cruise 63/20 Friday Harbor					
Surface	1.9, 2.1	7.7 –	8.3, 6.6	19.9, 25.7	30.5

TABLE X. Replicate samples from 20 m, station JF-1-1, cruise 63/20 (μg Fe/liter).

	Soluble		Particulate	
	HCl-reactive	Total	HCl-reactive	Total
	1.9	11.4	6.5	16.1
	1.9	15.4	6.4	17.1
	2.3	6.1	6.5	15.4
	2.7	9.0		
	3.2	15.3		
	2.2			
Mean:	2.4	11.4	6.5	16.2
SD:	±0.2	±4.0	±0.1	±1.1

TABLE XI. Comparison of iron values obtained by summing total particulate plus total soluble results with total iron in unfiltered samples (μg Fe/liter).

Cruise and station	Depth (*m*)	I Total Part.	II Total Sol.	III I+II	IV Total Unf.	V Δ(IV–III)
63/20 JF-1-3	20	6.4	5.8	12.2	10.2	−2.0
	20	6.6	4.8	11.4	13.5	+2.1
	50	10.6	4.8	15.4	21.5	+6.1
	50	14.5	21.5	36.0	64.1	+28.1
	75	11.5	6.5	18.0	19.2	+0.8
	75	11.8	3.9	15.7	14.2	−1.5
63/20 Geo-2-2	20	6.8	5.5	12.3	12.8	+0.5
	20	5.6	24.9	30.5	12.7	−17.8

TABLE XII. Seasonal and mean values for the discharge of iron from the Fraser River into the Strait of Georgia.[a]

	Collection date	River discharge[b] (km³/day × 10)	Total iron Concentration (μg Fe/liter)	Total iron discharge (kg/day × 10^{-5})
I- Agassiz				
	Aug. 9, 1962	3.15	1847	5.82
	Dec. 28, 1962	0.89	481	0.43
	June 8, 1963	4.30	952	4.09
II New Westminster				
	Aug. 9, 1962	3.15 × 1.26[c] = 3.97	2128	8.45
	Dec. 28, 1962	0.89 × 1.50 = 1.34	519	0.70
	June 8, 1963	4.30 × 1.24 = 5.33	983	5.24
III Annual Mean[d] *New Westminster*		2.00 × 1.36 = 2.72	751[e]	2.04

[a]The Fraser River discharge data was kindly supplied by the Department of Northern Affairs and National Resources, Ottawa.
[b]Values quoted for Hope were applied to iron results at Agassiz, 20 miles downstream, in order to include Dec. 28 samples, not taken at Hope.
[c]Correction factors to adjust discharge at Hope to discharge at New Westminster (from Waldichuk, 1957).
[d]River discharge for period Oct. 1, 1962 to Sept. 30, 1963.
[e]Mean of Dec. 28, 1962, and June 8, 1963.

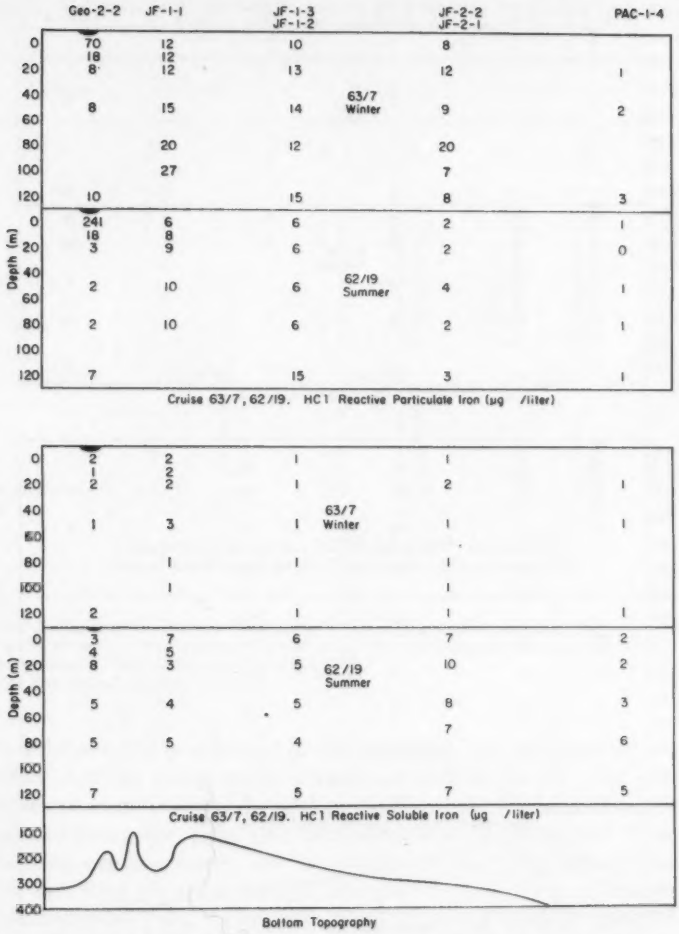

FIG. 3. Profile of HCl-reactive particulate and soluble iron from the Fraser River mouth out into the Pacific Ocean. The solid half circles represent the mouth of the Fraser River. Vertical exaggeration of bottom topography: 150×.

liberates a relatively constant fraction of the total particulate and soluble iron, which, as Strickland and Parsons (1960) suggest, may be a measure of iron more readily available to biological systems. The ratios derived from the river water samples are similar to those found for sea water even though the particulate and soluble iron concentrations in the river are several orders of magnitude higher.

The oceanographic data — temperature, salinity, dissolved oxygen, and a limited number of *p*H measurements — taken in conjunction with each

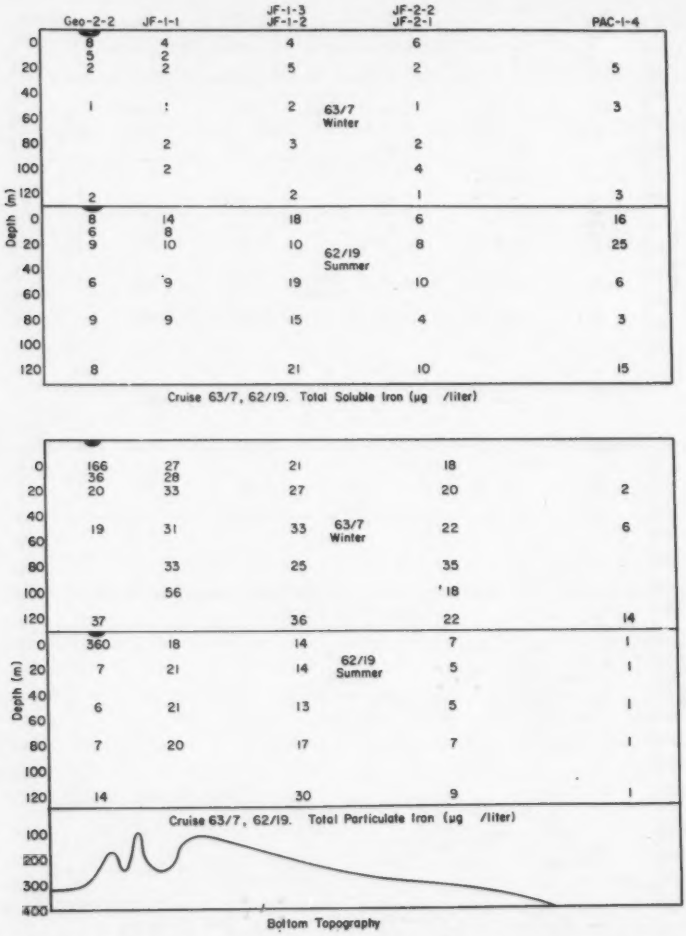

FIG. 4. Profile of total particulate and soluble iron from the Fraser River mouth out into the Pacific Ocean. The solid half circles represent the mouth of the Fraser River. Vertical exaggeration of bottom topography: 150×.

iron observation do not reveal any correlations. (These data are available as Data Reports, No. 21 and 23, 1962 and 1963, Institute of Oceanography, University of British Columbia.) The high particulate iron concentration in the low salinity surface water off the Fraser River was evident only in the Strait of Georgia and was not reflected in the surface waters beyond the mixing zone which occurs through the narrow sills in the San Juan Archipelago. Turbulent tidal flows through these passages render the shallow and deep waters nearly homogeneous (Waldichuk, 1957; Herlinveaux and Tully, 1961),

TABLE XIII. Ratios of total particulate iron to HCl-reactive particulate iron and of total soluble iron to HCl-reactive soluble iron.[a]

Station	Cruise:	62/16		62/19		63/7		63/20	
		P	S	P	S	P	S	P	S
Geo-2-1		1.8	3.5	–	–	–	–	–	–
Geo-2-2		2.7	(0.5)[b]	1.0	1.4	2.4	2.0	5.1	3.6
Geo-2-3		2.5	(0.9)	1.9	(0.6)	–	–	–	–
JF-1-1		–	–	2.4	1.9	2.1	1.0	2.9	4.2
JF-1-3		–	–	2.2	3.4	2.3	5.7	2.2	3.3
JF-1-2		–	–	2.2	2.4	2.3	3.7	–	–
JF-2-1		–	–	4.4	1.4	2.6	1.1	–	–
JF-2-2		–	–	2.3	(0.4)	1.5	2.5	–	–
Pac-1-4		–	–	2.0	2.7	3.8	12.4	–	–
	Date:	25/6/62		9/8/62		28/12/62		8/6/63	
Hope		–	2.3	2.8	5.0	–	–	2.7	2.0
Agassiz		–	1.4	1.4	3.0	2.9	2.2	1.3	3.4[c]
Mission		–	5.0	1.9	1.2	1.4	1.8	1.3	4.0
Langley		–	2.4	2.0	4.4	1.7	3.6	1.3	2.6
New Westminster		–	1.4	1.9	(0.6)	1.4	2.3	1.3	7.4
Steveston		–	2.7	–	–	1.0	1.5	1.2	2.9

Grand mean seawater: Particulate, 2.5[d]; soluble, 2.3[d]
Grand mean river water: Particulate, 1.7; soluble, 2.9

[a]Mean values for the entire water column. See text for fuller explanation.
[b]Results in parentheses represent lower total than HCl-reactive forms (see text).
[c]One extremely high value not included.
[d]Does not include station Pac-1-4.

and this homogeneity is reflected in the increased concentration of particulate iron throughout the entire water column at station JF-1-1. The gradual decrease in the concentrations of total particulate and HCl-reactive iron occurring seaward along the Juan de Fuca Strait can be accounted for from the influx of open oceanic water, low in particulate iron, and subsequent mixing. Since the sampling program was not designed to follow any particular period of the semidiurnal tidal cycle, and since sampling periods covered several days duration, there is no justification in relating the iron concentrations to any tidal excursions.

The total particulate iron values reported for the Fraser River (Table IV) are in general agreement with those of Durum et al. (1960), and are appreciably greater than the values reported by Leverin (1947). A comparison of these results is given in Table XIV for stations at Mission, New Westminster, and Hope. The HCl-reactive and total particulate iron concentrations increase going downstream from Hope to Agassiz, a distance of approximately 20 miles, and remain essentially constant from Agassiz to the mouth of the river. This increase reflects a larger sediment load acquired as the river broadens and scours greater areas of the Fraser River valley. Seasonal fluctuations in the discharge of total iron (Table XII) show that high iron concentrations

TABLE XIV. Comparison of total particulate iron values with previous work for the Fraser River (μg Fe/liter).

	Hope	Mission	New Westminster
Leverin (1947)			
26/7/38	30	80	–
4/12/38	70	–	–
19/8/42	–	–	210
Durum et al. (1960)			
7/1/58	–	1180	–
1/10/58	–	635	–
12/2/59	–	363	–
This paper			
9/8/62	710	2290	2118
8/6/63	624	915	954
28/12/62	–	430	495

and a greater water volume discharge combine to give ten times the total amount of iron entering the Strait of Georgia during summer than in winter. Soluble iron values, HCl-reactive, and total, remain essentially constant both seasonally and downstream from Hope to the Strait of Georgia. The HCl-reactive fraction may reflect either colloidal iron oxides or possibly a stable, soluble organic-iron complex which enters into the marine environment and which may persist in the open ocean.

ACKNOWLEDGMENTS

We wish to thank the Fisheries Research Board of Canada for use of the research vessel Investigator I, the Department of Mines and Technical Surveys for use of the research vessel C. S. S. *Ehkoli*, and the Royal Canadian Navy for use of the research vessel C. N. A. V. *Oshawa*. We are also grateful for the technical assistance provided by Messrs H. Wilke and T. Killam.

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In Vitro Cultivation of Cells of the Oyster, *Crassostrea virginica*[1,2]

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ABSTRACT

Cardiac tissue cells from oysters (*Crassostrea virginica*), cultured in a salt-enriched medium, apparently required a higher salt concentration for initiation of cell proliferation than that used for mammalian tissue cultures. The presence of human serum and bovine amniotic fluid enhanced the cell proliferation. Evidence of amitosis was observed in the cultured cells.

INTRODUCTION

The important role played by disease epidemics in oyster population dynamics has been demonstrated by the serious reduction of oyster stocks in Canada and the United States. The epidemics of one disease, "Malpeque," have been documented by Needler and Logie (1947), Logie (1957; MS, 1958), Logie et al. (1960), and Drinnan and Medcof (1961).

Studies of Malpeque disease have been hampered by an insufficient knowledge of the life history of the suspected infective agent, the inability to culture it, and the consequent incapacity to carry out controlled infection experiments. Conceivably, tissue culture techniques using oyster tissue could provide the tools required for these studies. The first requirement, before these techniques could be applied, was for a suitable medium and conditions for oyster cell proliferation.

In contrast to the cultivation of various tissues from land gastropods very little work has been done on the culturing of tissues from marine invertebrates. Tripp (1963) reported that the oyster heart explant can be cultured in hanging drop preparations with a medium consisting of sea water and animal sera. Perkins and Menzel (1964) stated that oyster cells can be maintained for periods of up to 42 days in a medium containing various oyster tissue extracts. They reported that there was no evidence of mitosis in these cells and gave only scant details regarding the morphology of the cultured cells.

[1]Received for publication September 2, 1965.
[2]Presented in part at the 14th annual meeting of The Canadian Society of Microbiologists, Fredericton, June 3–6, 1964.

J. Fish. Res. Bd. Canada, 23(4), 1966.
Printed in Canada.

This paper reports the effects of the major components of a culture medium on the initiation and maintenance of oyster cell proliferation, information on the morphology of the cultured cells together, and evidence that some of the cells divide by amitosis.

EXPERIMENTAL

The oysters (*Crassostrea virginica*) used were obtained from an area in Cape Breton, N.S., free of "Malpeque" diseases. The cardiac tissue of the oyster was removed under aseptic conditions and pooled lots of 5–8 organs were used for each experiment. Sterile scissors were used to mince the tissue in a salt-enriched basal medium (medium 1, Table I). Portions of the prepared tissue were transferred to plastic tissue culture flasks and, after the tissue became fixed directly to the flask surface (15–20 min), 1.5 ml of a test medium were added to each flask and the cultures were incubated at 18 C. Table I lists the composition of the six test media. All cultures were examined microscopically to estimate the extent of cell proliferation. The tissues for stained preparations were cultivated in Leighton tubes on 11 × 22-mm coverslips in the complete medium (medium 4), and then stained by the May-Greenwald-Giemsa or Feulgen technique (Merchant et al., 1960). The majority of the preparations were kept for longer than 6 days, a few for more than 2 weeks. After 2 weeks the cells in the different media were still alive, but were in the best condition in the complete medium (medium 4).

TABLE I. Compositions of the test media.[a]

Medium	Supplements[b]
1	50% SS[c]
2	50% SS + 10% BAF
3	50% SS + 10% HS
4	50% SS + 10% BAF + 10% HS
5	25% SS + 10% BAF + 10% HS
6	No SS + 10% BAF + 10% HS

[a]Basal medium contained medium 199, 5% bovine embryo extract, and 1% glutamine (200 mM). The following were used in all media: penicillin, 50 units/ml; streptomycin, 50 units/ml; and mycostatin, 25 units/ml. A concentrated medium 199 (Hank's base) was used, and the addition of the supplements was at the expense of the diluting agent (water) and thus would not affect the final concentration of medium 199 in the culture medium. The medium 199 and supplements were obtained from Becton, Dickinson and Company Canada Limited.

[b]SS, salt solution; BAF, bovine amniotic fluid; HS, human serum.

[c]Salt solution in grams/litre; NaCl, 18.67; KCl, 0.55; $CaCl_2$, 0.98; $MgCl_2 \cdot 6H_2O$, 5.00; $MgSO_4 \cdot 7H_2O$, 7.07.

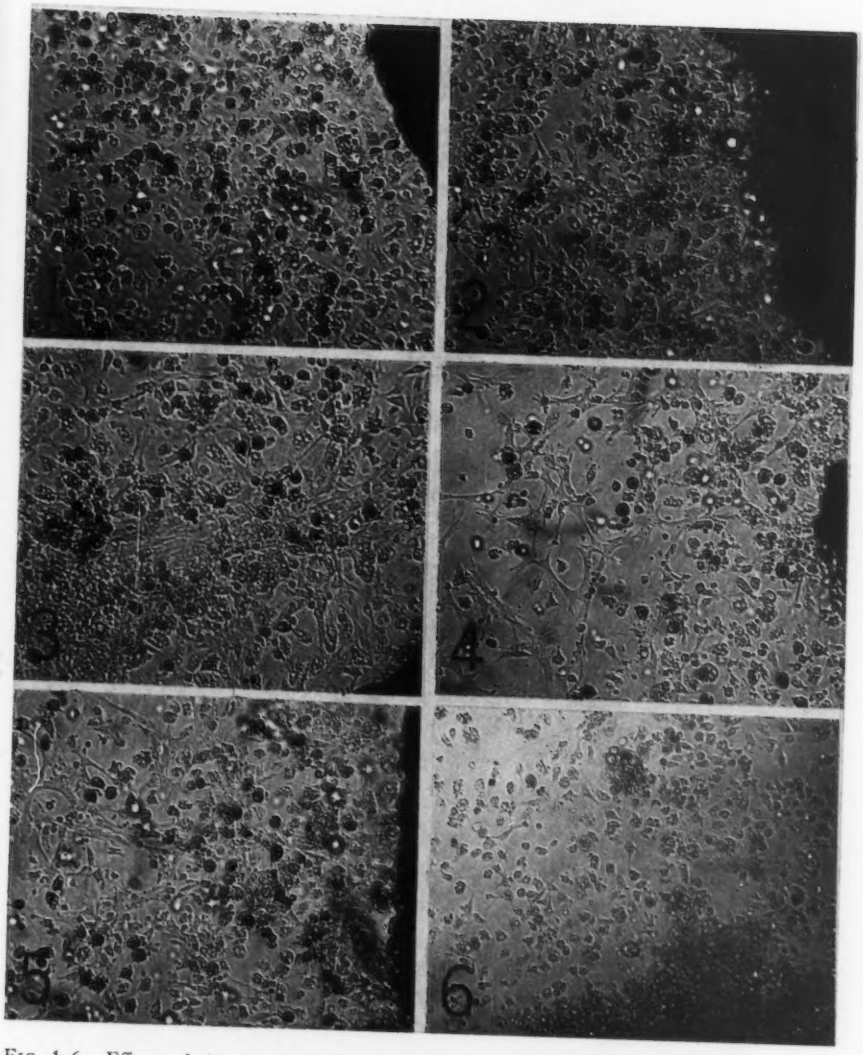

FIG. 1–6. Effects of the six test media listed in Table I on initiation of cell proliferation of oyster cardiac tissue. Photographed 3 days after the start of the experiment. Fig. 1–6, media 1–6, respectively. × 200.

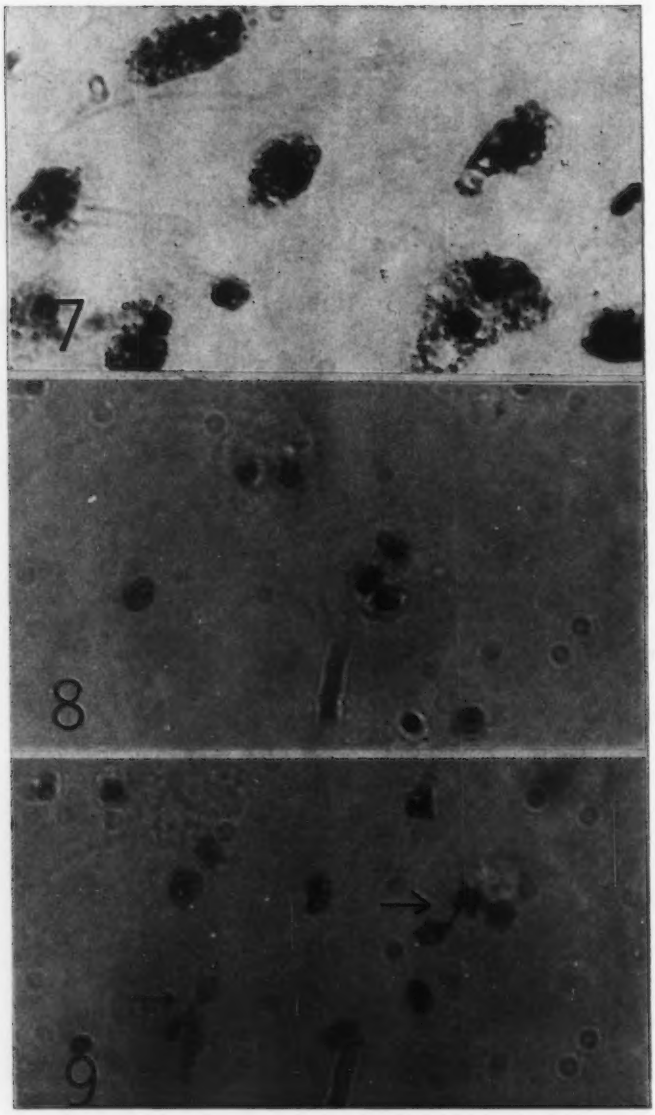

FIG. 7–9. Stained preparations of oyster cells cultured in the complete medium (medium 4) for 3 days. × 1260. **7.** Cells with multiple nuclei and vacuoles, May-Greenwald-Giemsa staining. **8–9.** Feulgen-stained preparations of oyster cells. **8.** Note the multinucleated cells. **9.** The arrows indicate cells with dividing nuclei.

RESULTS

The cell proliferation of the tissue explants varied from preparation to preparation. The relative response of the tissue to the test media, however, was practically constant. Table II shows results typical of the experiments.

Usually the cells from the prepared tissue were, within 24 hr or less, well attached around the edge of the explant and subsequently these cells started to proliferate during the next few days. The basal medium supplemented with 50% salt solution (medium 1) poorly supported cell proliferation and failed to maintain the proliferated cells, which declined rapidly after the first 2 days of the experiment. The response of the cells was markedly improved when the salt-enriched basal medium was supplemented with either 10% bovine amniotic fluid (medium 2) or 10% human serum (medium 3). Further

TABLE II. The cardiac tissue response.[a]

Medium	Age of culture in days[b]		
	2	4	6
1	+++	++	+
2	++++	+++	+++
3	++++	+++	++
4	++++	++++	++++
5	++++	++	+
6	+++	++	+

[a]Proliferation: + = poor; ++ = slight; +++ = moderate; ++++ = luxuriant.
[b]Approximately 50% of the test medium was removed and replaced with fresh medium at 2-day intervals.

improvement was gained by the addition of both supplements to the medium (medium 4). A reduction of the salt concentration caused a large decrease in cell proliferation (medium 5 and 6).

Pulsation of the explants usually started 2–3 days after preparation and some explants still pulsated after 2 months.

Photographs of a 72-hr culture in each test medium are shown in Fig. 1–6. The magnification in these photographs (× 200) was selected to portray the cultures as a whole, rather than the fine details of the individual areas. The majority of cells in the salt-enriched basal medium (medium 1, Fig. 1) were round and granular and cell proliferation was limited. The appearance of the culture in the medium supplemented with either bovine amniotic fluid, medium 2, or human serum, medium 3, indicates that the cells were actively

proliferating (Fig. 2, 3). The medium supplemented with both bovine amniotic fluid and human serum, medium 4, supported healthy, proliferating cells which migrated considerable distances from the explant (Fig. 4). The cell proliferation and cell migration declined in the medium with a reduced salt concentration (medium 5, Fig. 5). The culture in the medium from which the salt solution was omitted, medium 6, shows scattered cells and very little cell proliferation (Fig. 6).

When the 72-hr culture cells in the complete medium (medium 4) were stained by the May-Greenwald-Giemsa technique it was seen that the cells contained one or more nuclei and multiple vacuoles in the granular cytoplasm (Fig. 7). The multiple nuclei in the cultured cells are clearly demonstrated in the cells stained by the Feulgen technique (Fig. 8), and the Feulgen-positive nuclei dividing or branching away from the parent nucleus are shown in Fig. 9.

DISCUSSION

The addition of bovine amniotic fluid, human serum, and salt solution supplements to the basal medium is essential for the initiation and maintenance of oyster cell proliferation. The omission of either bovine amniotic fluid or human serum caused a decrease in cell proliferation, and the proliferated cell was supported poorly by the culture medium from which bovine amniotic fluid and human serum were both omitted. Although it is difficult to relate cell proliferation to specific compounds in a medium supplemented with numerous naturally occurring substances, both bovine amniotic fluid and human serum apparently affect the proliferation of oyster tissue cells.

A reduction of the salt concentration caused a decrease in cell proliferation, and little cell response was observed in the medium without salt supplementation. Apparently the oyster cell requires a higher salt concentration in its culture medium for proliferation than mammalian cells do. The salt concentration of the basal medium supplemented with 50% salt solution was similar to that of sea water (Robertson, 1964), so that the results are in agreement with the observation of Tripp (1963). He indicated that a better response by oyster tissue was observed in a 10% calf serum in seawater medium than in commercial vertebrate tissue culture media. However, according to our data the complete medium (medium 4) described above is superior to the 10% calf serum in seawater medium.

The majority of the cells observed in the cultures were granular, and similar to the leucocytes of *Ostrea edulis* described by Takatsuki (1934). The nucleus of the unstained cell was indistinguishable from the cytoplasm, but in the stained preparations the nucleus and the intracytoplasmic vacuoles were easily seen. In certain areas of the culture up to 5% of the cells contained two or more nuclei (Fig. 8) suggesting incipient amitosis; furthermore, dividing nuclei were also observed in culture (Fig. 9). Though Perkins and Menzel (1964) postulated that all cells found in monolayers had their origin in the

explant, our results suggest that at least a part of the population found in the culture originated from the migrated cells.

This finding is of considerable importance to our studies of the "Malpeque disease(s)." One of us (R.E.D.) found that the amoebocytes in the tissue taken from oysters affected by this disease contain one to four spherical bodies. The precise nature of these bodies has not been determined. The formation of multinucleated giant cells is not uncommon in some viral infections, and it is possible that the observed spherical bodies have been confused with multinuclei in the cells. Consequently the existence of multinuclei in the oyster cell should be taken into consideration when evaluating results especially in studies of viral infections.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the technical assistance of Mr S. C. Hazelden.

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Preservation of the Sardine *Sardinella melanura* by Low-Dose Gamma Radiation[1]

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ABSTRACT

Irradiating the sardine *Sardinella melanura* (Cuvier) with 15,000 or 23,000 rad from a cobalt source increased the storage life at 1 C in thermoregulated cabinets. During storage the rates of increase in *p*H, total volatile nitrogen (TVN), tyrosine value (TV), and bacteriological count (BC) were diminished by raising the radiation dose and those for TVN, TV, and BC also by treatment with chlortetracycline (CTC). The edibility periods, as judged organoleptically, were 4 days for untreated samples, 8 and 11 days for samples irradiated at 15,000 and 23,000 rad, and 12 days for samples treated with CTC. After 8 days' storage the TVN and TV values for samples treated with CTC were lower in relation to the values just after treatment than were those for samples irradiated at either dose. At 8 days, the BC values for samples treated with CTC were 136 times those just after treatment, in comparison with 11 times for the irradiated samples; however, the counts at 8 days were less than half as high for samples treated with CTC as for those irradiated at 23,000 rad.

INTRODUCTION

This is a report on using low doses of gamma radiation for fish preservation as compared with chlortetracycline treatment.

Irradiation of fishery products at doses of 50,000–2,000,000 rad has been shown to reduce the initial bacterial contamination and hence extend the useful storage life of the product by 12–84 days (Desrosier and Rosenstock, 1960; Eukel and Huber, 1960; Miyauchi, 1960; Masurovsky et al., 1963). Beta radiation as low as 11,800 rad has been shown to retard the spoilage of fish to a limited degree while beta radiation in the range of 30,000–50,000 rad has been reported to slow the onset of spoilage in a variety of fish and shellfish (Lerke et al., 1961). In countries where the population is increasing faster than the supply of protein-rich foodstuffs and where fish products are used by consumers within a short period after they are prepared, a short extension in the shelf life of the product might meet the technological and economic requirements. Further, use of low doses might avoid the undesirable chemical and organoleptic changes often caused by high rates of irradiation (Morgan and Siu, 1957; Desrosier and Rosenstock, 1960).

MATERIALS AND METHODS

The sardine *Sardinella melanura* (Cuvier) was taken as an experimental example for the common sardines of the Red Sea. Samples were caught at about 75 km south of the town of Suez in November and December 1963.

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J. Fish. Res. Bd. Canada, 23(4), 1966.
Printed in Canada.

Each catch, weighing about 150 kg, was kept at 1 C in thermoregulated transportable cabinets for 24 hr before experimentation and then divided into three groups. These were treated as follows:

The control group was kept at 1 C throughout the experiment. The second group was dipped in a solution of chlortetracycline (CTC) (15 ppm) for 5 min and then stored at 1 C. The third group was transported at 1 C to the Cobalt 60 Irradiation Facility at the UAR Atomic Energy Establishment, where irradiation with doses of 15,000 or 23,000 rad was carried out. The irradiated samples were then similarly stored at 1 C. During storage, all samples were packaged in sterilized polyethylene bags to prevent contamination from external sources.

The criteria used during the storage period after the treatments were: the hydrogen ion concentration (*p*H), the tyrosine value (TV), the total volatile nitrogen (TVN), and the bacterial count (BC). These criteria were considered the most suitable for judging the keeping qualities of fish by Riemann (1952), Ota and Ajiska (1953), and Naguib (MS, 1963). TV was expressed as milligrams of tyrosine per 100 g of flesh, TVN as milligrams of ammonia per 100 g of flesh, and BC as viable count per gram of flesh incubated at 30 or 20 C.

The *p*H (not taken from samples treated with CTC) was measured in the slurry of fish flesh and distilled water according to the recommendations of the Association of Official Agricultural Chemists (1950) using a meter with a glass electrode. The tyrosine values were estimated by the method of Naguib (MS, 1963), the phenol reagent being prepared according to Follin and Glocateu (1927). The tyrosine was determined in the clear filtrate using a colorimeter with a red filter (wavelength 6100 A). The total volatile nitrogen was determined by Okkoloff's magnesium oxide distillation method as cited by Winton and Winton (1947). The total bacterial count was made on the basal medium as given by Difco Laboratories (1948); the results were calculated according to the dilutions and the averge counts were recorded. The analyses were continued until organoleptic tests showed unacceptability.

In the organoleptic tests, samples for each treatment were examined for odor, texture, and appearance. They were described briefly as to their general condition and placed in one or other of five grades as shown in Table I. The gradings were made independently by at least three persons who knew nothing about the history of the fish.

The values obtained for TVN, TV, and BC were used to calculate ratios as follows: $\frac{\text{TVN (8 days after treatment)}}{\text{TVN (just after treatment)}} = \text{TVN } R_8$ and, similarly, TV R_8 and BC R_8. These ratios were taken as indices of the beneficial effects of the treatments.

The protection factor was calculated as follows: $\frac{\text{the storage life for treated samples}}{\text{the storage life for untreated samples}}$, the storage life being taken as lapse of time (in days) until the organoleptic tests showed unacceptability.

TABLE I. Storage behaviour of the sardine *S. melanura* at 1 C after being irradiated at two low levels of gamma rays or treated with chlortetracycline (CTC) as compared with untreated samples.

	Irradiation treatments															
	23,000 rad				15,000 rad				CTC treatment				Untreated samples			
Days of storage	*p*H	TVN[a]	TV[b]	Organoleptic grade[c]	*p*H	TVN	TV	Organoleptic grade	*p*H	TVN	TV	Organoleptic grade	*p*H	TVN	TV	Organoleptic grade
0	7.15	8.9	32.5	A	7.15	8.9	32.5	A	–	7.10	26.80	A	7.15	8.9	32.5	A
1	–	–	–	–	–	–	–	–	–	–	31.40	A	–	–	–	–
2	–	–	–	–	–	–	–	–	–	15.0	46.00	A	–	–	–	–
3	7.20	44.6	60.0	A	7.20	47.0	64.0	A	–	–	–	–	7.15	46.0	84.0	A
4	–	–	–	–	–	–	–	–	–	21.0	59.00	A	–	–	–	–
5	7.20	56.0	115.0	B	7.10	62.0	106.0	B	–	–	–	–	7.45	88.0	130.0	D
6	–	–	–	–	–	–	–	–	–	32.0	71.00	A	–	–	–	–
8	–	77.4	130.0	B	7.30	90.0	138.0	C	–	–	84.00	A	7.65	126.0	146.0	E
10	–	–	–	–	–	–	–	–	–	60.0	–	B				
11	7.40	83.0	151.0	C	7.55	120.0	180.0	E	–	–	–	–				
12	–	–	–	–	–	–	–	–	–	77.1	151.0	B				
13	7.60	125.0	167.0	E					–	–	–	–				
14									–	90.0	172.0	C				
16									–	105.0	180.0	C				
18									–	117.0	184.0	E				

[a]TVN: Total volatile nitrogen expressed as milligrams ammonia per 100 g flesh.
[b]TV: Tyrosine value expressed as milligrams tyrosine per 100 g flesh.
[c]A = fresh, B = normal, C = slight off-odor detected, D = doubtfully acceptable, E = unacceptable.

RESULTS AND DISCUSSION

Irradiation at each of the two rates had beneficial effects on the keeping quality of the sardines, though not so great as those from treatment with chlortetracycline.

The *p*H, TVN, and TV each increased in all cases during storage (Table I). The rates of increase were diminished by raising the radiation dose and, more markedly, by treatment with CTC.

Off-odor was detectable in all cases at a *p*H of 7.30 or higher and at TVN and TV values near 80 and 130, respectively. Thus the edibility period was 4 days for the untreated samples, 8 days for samples irradiated at 15,000 rad, 11 days for those irradiated at 23,000 rad, and 12 days for those treated with CTC.

In general, the bacterial count increased during storage (Table II). The rate of increase was diminished markedly by irradiation and somewhat by treatment with CTC. For the irradiated samples the count at the incubation temperature of 20 C was consistently greater than that at 30 C.

The protection factors were 2 and 2.75 for the irradiated samples and 3.0 for the CTC treatment (Table III). Though the BC R_8 for treatment with

TABLE II. Bacterial counts (in millions per gram of flesh after incubation) on the sardine *S. melanura* during storage at 1 C after being irradiated at two low levels of gamma rays or treated with chlortetracycline (CTC) as compared with untreated samples.

Days of storage	Irradiation treatments				CTC treatment		Untreated samples	
	23,000 rad		15,000 rad					
	30 C	20 C	30 C	20 C	30 C	20 C	30 C	20 C
0	0.65	2.4	2.4	2.5	0.025	–	3.2	2.9
1	–	–	–	–	0.09	–	–	–
2	–	–	–	–	0.5	–	–	–
3	0.34	0.32	10.5	11.6	–	–	43.0	22.0
5	5.0	8.7	16.5	46.0	–	–	227.0	47.0
6	–	–	–	–	1.8	–	–	–
8	7.5	10.9	26.0	91.0	3.4	–	490.0	890.0
10	22.0	64.0	87.0	164.0	4.2	–	–	–
12	26.0	147.0	115.0	214.0	4.0	–	5600.0	9400.0
14					5.3	–		
16					6.9	–		
18					7.5	–		

TABLE III. Ratios of deterioration during 8 days' storage at 1 C and the calculated protection factors for samples of the sardine *S. melanura* according to treatment.

Ratio[a]	Irradiation treatments		CTC treatment	Untreated samples
	23,000 rad	15,000 rad		
TVN R_8	8.7	10.1	6.4	14.1
TV R_8	4.0	4.2	3.1	4.4
BC R_8	11.5	10.9	136.0	153.0
Protection factor[a]	2.75	2.0	3.0	–

[a]For explanation of the ratios and the protection factor, see text.

CTC was much higher than for either of the irradiation treatments, the actual counts at 8 days after treatment were less than half as high for CTC as for irradiation (3.4 million vs. 7.5 and 26 million). That the BC R_8, which is a direct index of the rate of fish deterioration as a result of bacterial growth, was lower for irradiation than for treatment with CTC is in keeping with the findings of Ricotta (1962) that antibiotics have bacteriostatic effects while irradiation has bactericidal effects.

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NOTES

Foetal and Postpartum Whitecoat Pelage in *Phoca vitulina*

Except in *Phoca vitulina largha* of the western Pacific Ocean and Bering Sea (Ognev, 1935; Schwarz, 1942) harbour seal pups are normally born in a spotted coat of essentially adult character (Scheffer and Slipp, 1944; Montagna and Harrison, 1957). *Phoca vitulina largha* differs from all other subspecies of *P. vitulina* by pupping on floating ice and bearing a whitecoat pup (Ognev, 1935; Wilke, 1954; King, 1964). The harbour seals of the Atlantic, *P.v. vitulina* and *P.v. concolor*, and *P.v. richardi*, of the North American Pacific coast, avoid both open ocean and ice packs (Ognev, 1935; Dunbar, 1949) and normally bear their pups on shore in areas where incoming tides submerge the sites (Ognev, 1935; Scheffer and Slipp, 1944; Fisher, 1952; King, 1964).

Pre-natal and neo-natal pelage. In Washington State, Scheffer and Slipp (1944) noted that the yellowish-white foetal or lanugo pelage was usually replaced by a short spotted coat prior to parturition. Montagna and Harrison (1957) wrote that the common seal of the Wash shed its "wooly juvenile fur" *in utero*. Bonnot (1951) stated that, in California, the pre-natal coat was shed before birth. Ognev (1935) stated of *P.v. vitulina* that "whitelings are never encountered among common seals." Scheffer (1958) asserted that there seemed to be no record of *P.v. richardi* newborn young in the embryonic pelage. Exceptions do occur. King (1964) commented that the embryonic hair was occasionally shed immediately after birth. Fisher (1952) reported finding a pup, on the Ecstall River, B.C., which still possessed an exceedingly loose coat of foetal hair some 15–20 min after parturition. In 1965, lanugo hair was noted on post-natal pups at Humboldt Bay, California, on May 10; Boundary Bay, B.C., on July 23, Victoria, B.C., on August 6; and Port Moody, B.C. (Fig. 1.), on August 10.

Comparison of tanned skin samples from a *P.v. largha* whitecoat pup (Fig. 2) collected in the Bering Sea and a *P.v. richardi* whitecoat foetus from British Columbia (both skins from Hansen Fur Company, Langley, B.C.) indicates that the greater thickness and woolier texture of the *P.v. largha* pelage reflect differences in the hair of the two forms. Under the binocular microscope the lanugo hair of *richardi* (Fig. 3A) appeared composed of straight overhairs surrounded and interspersed with much thinner underhairs. The overhairs appeared about five times the diameter of the underhairs. The longest hairs measured 20 mm in length. In *largha* (Fig. 3B) the hairs were generally wavy. The overhairs were about half the diameter of the corresponding hairs in the embryonic coat of *richardi*. The underhairs were about one-fifth the diameter of the overhairs and nearly as long. The longest hairs

J. Fish. Res. Bd. Canada, 23(4), 1966.
Printed in Canada.

measured 30 mm. In both subspecies the hairs appeared milkily opaque and without visible pigment.

I have examined approximately 100 *P.v. richardi* foetal skins. These fell roughly into two categories. Firm hair, "dirty" white or pale yellowish-grey, with a faintly darker area along the dorsal midline, which resisted tugging and remained on the pelt appeared characteristic of a foetus at about half term. As the foetus neared parturition the hair would become progressively looser until it could be scraped with my thumb from the skin. Near-term foetal skins rarely exhibited any lanugo hair.

Moult of the embryonic hair both *in utero* and when post-natally present follows a regular pattern in *P.v. richardi* as determined by examination and photography of a series of samples. Such a series has not been available for *P.v. largha*. In *richardi* the lanugo hair begins to shed on the anterior dorsal surface. As the moult continues in the anterodorsal area the posterior dorsal region begins to shed. The moult is completed on the anterior and posterior dorsal surfaces and the flanks before the foetal hair is shed from the ventral region. The mid-dorsal line posterior to the pectoral flippers is usually the final area to moult the foetal hair in *richardi*. In both *richardi* and *largha* the foetal hair initially conceals the post-natal coat which is exposed by moult or removal of the lanugo hair (Fig. 2).

The possible adaptive significence of either *in utero* or post-natal shedding is suggested by the development of geographic variations within a widespread species having rather weakly separable subspecies (Ognev, 1935; Scheffer, 1958). White hair colour might have some protective function if newborn harbour seals, like newborn harp seals (*Pagophilus groenlandicus*) were at least temporary nonswimmers who were exposed on ice (Ognev, 1935; Fisher, 1955; King, 1964). Fisher (1952) commented on the ability of a newborn harbour seal to swim. He also noted that a pup born on an Ecstall River sandbar, in an area where almost all postpartum pups wear the spotted coat, was nearly invisible on the sand because it possessed a matted coat of yellowish-white lanugo hair (Fisher, 1952). The conditions of nonswimming pups exposed on ice are not met in any harbour seal subspecies except *P.v. largha* (Ognev, 1935; Wilke, 1954; King, 1964). Wilke (1954) working off Hokkaido with Japanese sealers found that *P.v. largha* pups were born on floating ice between late January and mid April and were subject to predation while they were on the ice. These pups were in various stages of moult in late April.

The presence of a white pre-natal coat in all the races and the occasional birth of white pups in *P.v. richardi* as far south as California indicate that white post-natal pups may once have been universal in *P. vitulina* stocks. It is probable that *P.v. largha* during the Pleistocene was long separated from the other races of *P. vitulina* and retained its white juvenile post-natal pelage due to retention or adoption of pack-ice pupping areas where the whitecoat pup has a survival advantage over the coloured pup.

The recently described sympatric species or subspecies *P.* (*vitulina*?) *insularis* (Belkin, 1964) of the Kurile Islands bears a spotted pup on shore

FIG. 1. *Phoca vitulina richardi* female pup captured at Port Moody, B.C., on August 10, 1965. Weight 17 lb. Umbilicus 3½ inches long, pink-red. Foetal (lanugo) hair coat prominent except on top of head, face, and foreflippers. Photographed at service yard, Stanley Park, Vancouver, B.C., August 13, 1965.

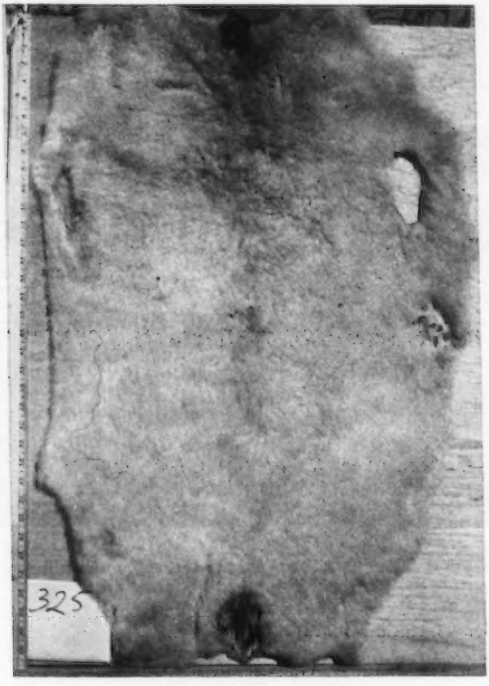

FIG. 2. Tanned skin of *Phoca vitulina largha* pup from Bering Sea. Depth of the white wooly coat 30 mm. Note the short, spotted adult-type coat visible in the three small plucked areas.

FIG. 3. Tanned skin slices viewed in cross section. Magnification about 3 ×. A. *Phoca vitulina richardi* foetus. B. *Phoca vitulina largha* whitecoat pup.

in May, is reproductively isolated from *P.v. largha*, and seems much more like harbour seals in general. The observed differences in the whitecoat hair of *P.v. richardi* and *P.v. largha* plus the existence of *Phoca "insularis"* indicate that if *P.v. largha* is indeed a subspecies of *P. vitulina* it is a very different seal from the rest of the species.

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Received for publication September 19, 1965.

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Description of Groundfish Trawl Developed for the Side-Trawl Research Vessel *G. B. Reed*

Prior to acquisition of the 177-ft research vessel *G. B. Reed* in December 1962, trawling operations of this Station were conducted aboard small stern-trawlers 60–80 ft in length. For groundfish operations using the *G. B. Reed*, a side trawler, it was necessary to construct a groundfish trawl suitable for use aboard this vessel. Accordingly, Captain J. F. L. Parker adapted a commercial otter trawl design to develop the trawl described in this report. It was constructed primarily for catching Pacific ocean perch (*Sebastodes alutus*) and has proved satisfactory to date for this purpose.

Synthetic fibres (multi-filament polypropylene twine) are used throughout in construction of the net. Nylon net (Drumlene) treated with a proprietary tar mixture has been used for the codend liner. The specifications of the various materials used are:

Trade name of material	Diameter (*inches*)	Tensile strength (*lb*)	Mesh size[a] (*inches*)
18-cord Gunlene (orange)	0.115	250	4.5
No. 60[b] Drumfil (green)	0.135	326	4.5
No. 96[b] Drumfil (green)	0.175	568	3.0
No. 9 Drumlene (black)	0.083	96	1.5

[a]Internal mesh size (stretch measure between knots).
[b]The number ("60" and "96") is the same as used for cotton twine and indicates material of similar gauge.

Net and liner. For the first net constructed, the wings, square section, and belly were made from 18-cord Gunlene. In all subsequent nets Gunlene was replaced with No. 60 Drumfil. Intermediate and codend sections are constructed of No. 96 Drumfil, the codend being double knit to provide greater strength. To inhibit abrasion, cowhide chafing gear is laced to the underside of the codend. A splitting strap made of galvanized-wire rope, 0.5-inch in diameter, is located 20 meshes from the after end of the codend.

Two quarter ropes (polypropylene, 1.125-inch diameter) are necessary in side trawling to close the net as it approaches the vessel, and assist in hauling the groundrope aboard. The 16-fath left quarter rope (port side of net underway) is fastened to the groundrope 10 ft left of center. It passes around the outside of the net, through a becket 10 ft left of center on the headrope, and is secured to the left danleno assembly for retrieval when closing the net. The 14-fath right quarter rope (starboard side of net underway), which is fastened to the groundrope 10 ft right of center, passes around the outside of the net, through a becket 10 ft right of center on the headrope, and is secured to the right danleno assembly.

J. Fish. Res. Bd. Canada, 23(4), 1966.
Printed in Canada.

The total length of the net is 119 ft (375 meshes). Dimensions and cutting angles are given in Fig. 1. No rib lines are used; all sections are joined together with Drumfil twine, whose size matches that of the meshes to be joined.

When juvenile fish are sought, a codend liner (1.5-inch mesh) made of No. 9 Drumlene is installed. The liner, which measures 256 meshes in circumference by 400 meshes in depth, is hung on a 16-ft length of 0.5-inch diameter manila rope. The rope is then laced to meshes in the lower intermediate. This arrangement facilitates installation and removal.

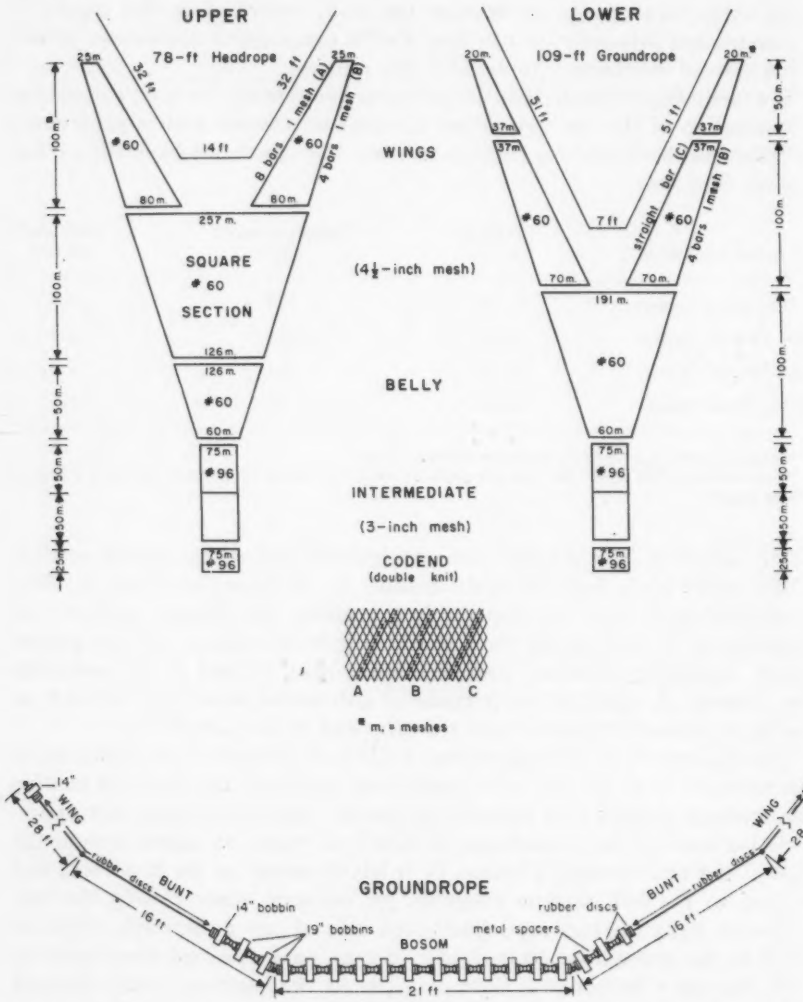

FIG. 1. Plan of otter trawl showing net measurements, cutting angles, and groundrope layout.

Headrope and footrope. The 78-ft headrope, to which the leading edge of the square section and the upper edge of each wing are hung (about 18 meshes/fath of headrope), is constructed of braided, nylon-covered, 0.5-inch diameter, wire rope in three 26-ft sections shackled together. Spare sections are stored aboard ship for quick replacement of damaged sections. Thirty-five 8-inch "deep-sea" aluminum floats[1] are attached to the headrope. Twenty-one are spaced evenly along the center section, and seven spaced along each wing section.

The footrope, to which the leading edge of the belly and lower edge of each wing are hung (about 17 meshes/fath of footrope), is 117 ft long, and is a single length of 0.5-inch diameter polypropylene rope.

Fishing line and groundrope. The 114-ft fishing line, which is lashed to the footrope at 2-ft intervals, consists of five sections shackled together: one 22-ft bosom section, two 17-ft bunt sections, and two 29-ft wing sections. It is constructed of 0.75-inch diameter, manila-wire rope (six-strand manila, each strand with a wire rope core).

The groundrope is 109 ft long, and consists of five sections of 6 × 24 galvanized wire rope (0.875-inch diameter) shackled together (Fig. 1). Evenly spaced along the 21-ft bosom section are twelve 19-inch diameter rubber bobbins, each separated by a metal spacer and two or more 7-inch diameter rubber discs. On the inward end of each 16-ft bunt section are two, 19-inch, rubber bobbins and one, 14-inch rubber bobbin, each separated by a metal spacer and rubber discs as on the bosom section. Seven-inch rubber discs cover the remaining length of each bunt section. The 28-ft wing sections are also covered with 7-inch rubber discs, and a 14-inch rubber bobbin is located at the extreme outward end of each section. Sixteen-inch lengths of chain suspend the groundrope from the fishing line: one length to each metal spacer, and one at each 3-ft interval along the remaining bunt and wing sections.

Danleno assembly and otter boards. Each danleno assembly has a 21-inch-diameter steel bobbin and a butterfly connected to the headrope by a 9-ft length of 6 × 24 galvanized wire rope (0.875-inch diameter). A 10-ft length of 6 × 24 galvanized wire rope (1.0-inch diameter) connects the butterfly to the groundrope. A 30-fath ground cable of 6 × 24 galvanized wire rope (0.875-inch diameter) connects the danleno assembly to the Brompton otter board, which measures 10.5 ft by 4.5 ft and weighs 1400 lb.

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Received for publication August 17, 1965.

[1]Diagrams and descriptions of various standard components may be found in the catalogue Trawling Equipment, issued by the Great Grimsby Co. Ltd. of Grimsby, England. These components include the aluminum floats, rubber bobbins, metal spacers, rubber discs, danleno assembly, and Brompton otter boards.

1α-Hydroxycorticosterone from Cartilaginous Fish: a New Adrenal Steroid in Blood[1]

Recent studies of corticosteroids in the peripheral blood of elasmobranchs required the purification and analysis of extracts of the blood of two species of *Raja* — *radiata* Donovan and *ocellata* Mitchill. Thin-layer chromatography (TLC), in contrast to paper, had the capacity to separate the more polar corticosteroid-type compounds from the large amounts of lipid extracted by chlorinated-hydrocarbons from skate plasma. However, extracts of plasma gave no evidence of the expected corticosteroids, cortisol, and corticosterone. Chester Jones and Phillips (1960) reported 8 μg/100 ml of corticosterone in the peripheral plasma of *R. clavata* and 5.3 μg/100 ml of cortisol in *R. eglanteria*, i.e. concentrations that would be clearly evident on "phosphor treated," silicic acid chromatoplates. Subsequently, double isotope analyses indicated that both cortisol and corticosterone were present in our plasma samples at concentrations of approximately 0.1 μg/100 ml. Chromatoplates consistently revealed the presence of an unknown compound more polar than cortisol. Preliminary chemical tests showed this compound to possess the functional groups essential to biological activity. It was difficult to isolate a sufficient quantity of the polar unknown (*I*, Fig. 1) from blood samples for identification but later it was obtained from interrenal incubates, and finally, corticosterone was found to be an efficient precursor.

A media developed by Nandi and Bern (1965) for the incubation of tissue from teleosts had to be modified in order to maintain a steady *p*H during the incubation of sectioned skate interrenals. Satisfactory results were obtained with 11.19 g NaCl, 0.373 g KCl, 0.333 g $CaCl_2$, 0.407 g $MgCl_2 \cdot 6H_2O$, 0.42 g $NaHCO_3$, 0.069 g $NaH_2PO_4 \cdot H_2O$, 1.08 g glucose, and 1.5 g Tris, *p*H 7.4 buffer per liter. Interrenal tissue (855 mg) from mature male *R. radiata* was preincubated for 45 min in 18 ml of media containing 1 unit of porcine ACTH per milliliter. The tissue was then added to 18 ml of fresh media containing 660 μg of corticosterone, 0.2 ml propylene glycol, 5 mg NADP, 20 mg glucose-6-phosphate, and 20 units of ACTH. The flask was flushed with O_2, stoppered and incubated in a Dubnoff metabolic shaker at 24 C for 5 hr. Co-factors were replenished at 1.5 hr and the system was flushed with O_2 after 1.5 and 3 hr. Steroids were extracted from the media with dichloromethane and fractionated by thin-layer chromatography on silicic acid with $CHCl_3$:CH_3OH:H_2O (90:10:1). The yield of *I* was 433 μg (63%). Incubation of *R. radiata* glands with [4-^{14}C] corticosterone demonstrated that conversion of corticosterone to *I* could be essentially quantitative although small amounts of a compound (UV-absorbing, blue-tetrazolium positive) with similar chromatographic

[1]Details of this study will be presented at the 2nd International Congress on Hormonal Steroids. Milan, Italy, May 1966.

J. Fish. Res. Bd. Canada, 23(4), 1966.
Printed in Canada.

mobility to 11-dehydrocorticosterone was formed. Actual recoveries of *I* depended upon tissue: precursor ratios. Interrenal tissue (410 mg) from mature female *R. ocellata* produced 410 μg (59%) of *I* in 6 hr at 18 C and 578 μg (83.5%) overnight after fresh media and substrate (660 μg) were added. A total of ca. 200 interrenals were incubated for varying lengths of time at various temperatures (ambient to 30 C) in the presence of different amounts of substrate and co-factors, and with and without the addition of antibacterial and antifungal agents. None of these factors appeared to exert a significant qualitative effect on the metabolism of corticosterone and *I* was always the major product.

I from interrenal incubates had the following properties: on Whatman No. 1 paper an $R_{cortisol}$ of 0.62 was obtained in benzene:methanol:water (2:1:1) after 18 hr at 25 C; it reacted immediately with blue-tetrazolium (α-ketol); it had $\lambda_{max}^{methanol}$ 242.5 mμ (Δ^4-3-ketone); it reacted rapidly with isonicotinic acid hydrazide (INH) (Δ^4-3-ketone); it gave a negative Porter-Silber reaction (no dihydroxyacetone or 16 OH group); it reacted quantitatively with 20β-hydroxysteroid dehydrogenase (20-carbonyl); it exhibited the same quantitative acid fluorescence in H_2SO_4:C_2H_5OH (70:30) as corticosterone; in conc H_2SO_4 after 2 hr λ max were 241, 286, 379, and 463 mμ and the relative OD values were 1.5, 3.6, 1.1, and 1.0.

Figure 1 illustrates the chemical transformation of the steroid from skate *I* to Δ^1-corticosterone *VI* and other derivatives. Treatment of *I* with acetic anhydride:pyridine (20:1) at 37 C for 16 hr gave *II* as the major of

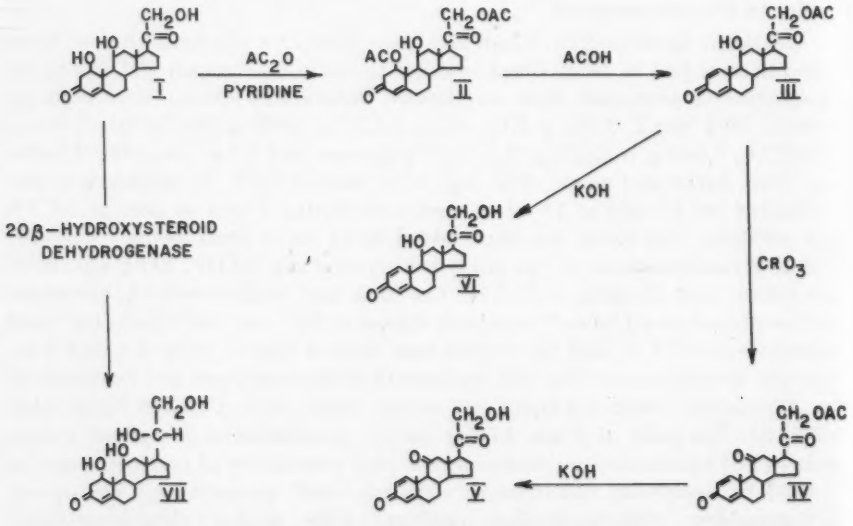

FIG. 1. Transformations of 1α-hydroxycorticosterone. *I*: 1α-hydroxycorticosterone; *II*: 1α-hydroxycorticosterone-1,21-diacetate; *III*: Δ^1-corticosterone-21-acetate; *IV*: Δ^1-11-dehydrocorticosterone-21-acetate; *V*: Δ^1-11-dehydrocorticosterone; *VI*: Δ^1-corticosterone; *VII*: 1α-hydroxy-20β-dihydrocorticosterone.

three products isolated by TLC. One hydroxyl acetylated quantitatively (21), another was almost completely acetylated (1α), and a third was very refractory (11β). *II* had an $R_{\text{corticosterone acetate}}$ of 0.93 following paper chromatography with heptane:benzene (1:1)–70% methanol (HB-70). Infrared spectra of *IV* showed no evidence of a hydroxyl group (no OH at carbons 8, 9, or 14).

Chromatographic mobilities on paper of authentic samples established that the additional hydroxyl of *I* had not been added to corticosterone at 2β, 6β, 12α, 15β, 18 or 19. The 2β and 6β positions were also eliminated since *I* did not react with zinc-acetic acid. Polarity considerations eliminated the 6α- and 15α-epimers and 2α-hydroxycorticosterone, prepared by alkali isomerization of the 2β-epimer, was less polar than *I*. There was no evidence in the infrared or ultraviolet spectra for a 4 OH group. The spectra of *I* in dilute alkali eliminated the 7β OH. Consideration of data available to this point favoured the 12β or 1 positions. The NMR spectra (Table I) established that the hydroxyl group could be only in the 1α-position (Tori and Kondo, 1964). It remained to convert *I* to a known compound.

TABLE I. Proton magnetic resonance spectra. Chemical shifts[a] of protons attached to carbon atoms at positions 1, 4, 11, 18, 19 in corticosterone, 1α-hydroxycorticosterone (*I*) and derivatives.

Steroid	19	18	4	11	1
Corticosterone	8.56	9.06	4.33	5.60	
I	8.55	9.05	4.25	5.45	5.63
Corticosterone monoacetate	8.50	9.07	4.34	5.56	
I diacetate	8.49	9.05	4.25	5.88	4.58

[a] τ values were determined in deuterochloroform with tetramethylsilane as an internal reference. The PMR spectra were recorded on a Varian A60 NMR spectrometer equipped with a C 1024 computer of average transients.

When *II* was refluxed for 1 hr in glacial acetic acid a single major UV absorbing product *III* was formed with an $R_{\text{corticosterone acetate}}$ of 0.90 following paper chromatography in HB-70. It reacted very slowly with INH a property characteristic of $\Delta^{1,4}$-3-ketones. *III* was also prepared from authentic Δ^{1}-corticosterone as described above and the mobilities of both acetates were identical by TLC in benzene:EtOAC (4:6) and EtOAC:$CHCl_3$:H_2O (90:10:1). *III* was converted to *IV* by treatment with CrO_3 in acetic acid for 20 min at 20 C. *IV* was hydrolyzed with 0.07 N alcoholic KOH under N_2 for 2 hr to yield *V*. *V* from interrenal incubates and the authentic compound were chromatographed in HB-70 and had $R_{\text{corticosterone}} = 1.22$. *III* was hydrolysed to *VI* as above and had $R_{\text{corticosterone}} = 0.66$ in HB-70. *VI* from interrenal incubates and the authentic compound had $\lambda_{\text{max}}^{\text{methanol}}$ 243 mμ; $\lambda_{\text{max}}^{\text{KBr}}$ 2.82 and 2.90–2.96 mμ (OH); 5.85 μ (20-carbonyl); 6.06, 6.21, 6.25, and 11.34 μ ($\Delta^{1,4}$-diene-3-one).

I was isolated from 1 liter of blood obtained from fish of both sexes of *R. radiata* and established to be identical with *I* from interrenal incubates by conversion to *II*, *III*, and *IV*. All blood samples analyzed contained a substance

with the chromatographic mobility of *I* and were variously estimated to contain 0.5–2 μg/100 ml.

To the authors' knowledge 1α-hydroxycorticosterone has neither been isolated from a natural source nor synthesized. 1α-Hydroxylation has been seldom reported microbiologically (Dodson et al., 1957).

Δ^1-9α-Fluorocortisol has both intense glucocorticoid and mineralocorticoid activity but 1ξ-hydroxy-9α-fluorohydrocortisol (probably 1α), while still active as a sodium-retaining hormone, is "virtually inactive" in the liver glycogen assay (McAleer et al., 1958).

It has been suggested that the biosynthetic pathways in mammalian adrenocortical tissue are common to all vertebrates (Phillips and Bellamy, 1963). Such a statement seems premature since only a few of the thousands of species of fish have been investigated. There need only be a single modification or quantitative difference in a biosynthetic pathway to provide an animal with a different major blood or tissue hormone. Thus, cortisone rather than cortisol can at times be the principal hormone in fish blood and testosterone, 17α-hydroxyprogesterone, and other steroids can occur at extremely high levels. 11-Ketotestosterone, as yet unknown in mammals, can be a principal blood hormone in fish where it functions as a potent androgen (Schmidt and Idler, 1962). When one considers the special problems presented to an animal living in a marine environment, and particularly to an anadromous species, there is good reason to believe that many more novel quantitative and some qualitative differences will be found in hormones in the blood and tissues of fish.

It is interesting to speculate that the 1-hydroxyl group could serve as a potential precursor of the extremely biologically active $\Delta^{1,4}$-diene-3-one structure.

Elasmobranchs have interesting ionic mechanisms to regulate, such as urea retention and sodium excretion via the rectal gland. It would seem worthwhile to determine whether the new hormone plays a role in such processes.

NMR spectra were determined and interpreted by A. G. McInnes and D. G. Smith, National Research Council, Halifax, N.S. Samples of commercially unavailable steroids for reference standards were kindly supplied by Drs S. Bernstein, M. Ehrenstein, W. Klyne (MRC collection), R. Neher, J. Pasqualini, F. G. Péron, B. Riegel, J. J. Schneider, M. Shirasaka, D. Taub, and N. L. Wendler.

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Received for publication March 2, 1966.

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